No. 2022-1461

In the United States Court of Appeals
For the Federal Circuit

BAXALTA INCORPORATED, BAXALTA GMBH,
Plaintiffs - Appellants

v.

GENENTECH, INC.,
Defendant - Appellee

Appeal from the United States District Court for the District of Delaware
No. 1:17-cv-00509, Hon. Timothy B. Dyk

BRIEF OF APPELLANTS

MORGAN, LEWIS & BOCKIUS LLP
Michael J. Abernathy
Christopher J. Betti
Maria E. Doukas
Karon N. Fowler
110 North Wacker Drive
Chicago, IL 60606
(312) 324-1000

MORGAN, LEWIS & BOCKIUS LLP
William R. Peterson
1000 Louisiana St., Ste. 4000
Houston, TX 77002
(713) 890-5188

MORGAN, LEWIS & BOCKIUS LLP
Julie S. Goldemberg
1701 Market Street
Philadelphia, PA 19103
(215) 963-5095

Counsel for Appellants

CLAIMS 1, 3, 4, AND 19 OF U.S. PATENT NO. 7,033,590

1. An isolated antibody or antibody fragment thereof that binds Factor IX or Factor IXa and increases the procoagulant activity of Factor IXa.

3. The antibody or antibody fragment according to claim 1, wherein the antibody is an IgG, IgM, IgA or IgE antibody.

4. The antibody or antibody fragment according to claim 1, wherein said antibody or antibody fragment is selected from the group consisting of a monoclonal antibody, a chimeric antibody, a humanized antibody, a single chain antibody, a bispecific antibody, a diabody, and di-, oligo- or multimers thereof.

19. The antibody or antibody fragment according to claim 4, wherein the antibody is a humanized antibody.

CERTIFICATE OF INTEREST

Case Numbers	2022-1461
Short Case Caption	*Baxalta, Inc. v. Genentech*
Filing Party/Entity	Appellants Baxalta Inc. and Baxalta GmbH

I certify the following information and any attached sheets are accurate and complete to the best of my knowledge.

/s/ William R. Peterson
William R. Peterson
Counsel for Appellants,
Baxalta Inc. and Baxalta GmbH

Dated: June 10, 2022

1. Represented Entities. Fed. Cir. R. 47.4(a)(1).	**2. Real Party in Interest.** Fed. Cir. R. 47.4(a)(2).	**3. Parent Corporations and Stockholders.** Fed. Cir. R. 47.4(a)(3).
Provide the full names of all entities represented by undersigned counsel in this case.	Provide the full names of all real parties in interest for the entities. Do not list the real parties if they are the same as the entities. ☑ None/Not Applicable	Provide the full names of all parent corporations for the entities and all publicly held companies that own 10% or more stock in the entities. ☐ None/Not Applicable
Baxalta Incorporated	Not Applicable	Takeda Pharmaceutical Company Limited
Baxalta GmbH	Not Applicable	Takeda Pharmaceutical Company Limited

4. Legal Representatives. List all law firms, partners, and associates that (a) appeared for the entities in the originating court or agency or (b) are expected to appear in this court for the entities. Do not include those who have already entered an appearance in this court. Fed. Cir. R. 47.4(a)(4).

Jody C. Barillare (Morgan, Lewis & Bockius LLP)	Wan-Shon Lo (Morgan, Lewis & Bockius LLP)	Jason C. White (Morgan, Lewis & Bockius LLP)
Amy M. Dudash (Morgan, Lewis & Bockius LLP)	Maria E. Doukas (Morgan, Lewis & Bockius LLP)	Amanda S. Williamson (Morgan, Lewis & Bockius LLP)
Margaret A. McGreal (Morgan, Lewis & Bockius LLP)	Olga Berson (Morgan, Lewis & Bockius LLP)	Caroline Lourgos (Formerly of Morgan, Lewis & Bockius LLP)
Colm F. Connolly (Formerly of Morgan, Lewis & Bockius LLP)	Jennifer M. Dienes (Formerly of Morgan, Lewis & Bockius LLP)	Jesse T. Dyer (Formerly of Morgan, Lewis & Bockius LLP)
Sanjay K. Murthy (Formerly of Morgan, Lewis & Bockius LLP)	Jessica A. Stow (Formerly of Morgan, Lewis & Bockius LLP)	

5. Related Cases. Provide the case titles and numbers of any case known to be pending in this court or any other court or agency that will directly affect or be directly affected by this court's decision in the pending appeal. Do not include the originating case number(s) for this case. Fed. Cir. R. 47.4(a)(5). See also Fed. Cir. R. 47.5(b).

None

6. Organizational Victims and Bankruptcy Cases. Provide any information required under Fed. R. App. P. 26.1(b) (organizational victims in criminal cases) and 26.1(c) (bankruptcy case debtors and trustees). Fed. Cir. R. 47.4(a)(6).

☑ None/Not Applicable

TABLE OF CONTENTS

Page

TABLE OF CONTENTS
(continued)

Page

TABLE OF CONTENTS
(continued)

Page

TABLE OF AUTHORITIES

Page(s)

CASES

TABLE OF AUTHORITIES
(continued)

Page(s)

TABLE OF AUTHORITIES
(continued)

Page(s)

STATUTES

TABLE OF AUTHORITIES
(continued)

Page(s)

RULES

OTHER AUTHORITIES

STATEMENT OF RELATED CASES

This case was the subject of a previous appeal to this Court. *Baxalta Inc. v. Genentech, Inc.*, No. 2019-1527. The panel comprised then-Judge Moore and Judges Plager and Wallach. The case was decided on August 27, 2020, and the opinion is reported at 972 F.3d 1341.

Pursuant to Federal Circuit Rule 47.5(b), counsel are not aware of any other cases pending in this or any other court or agency that will directly affect or be directly affected by this Court's decision in the pending appeal.

STATEMENT OF JURISDICTION

The district court had jurisdiction under 28 U.S.C. § 1338(a). The district court entered a final judgment in favor of Genentech on January 13, 2022. Appx77. Baxalta filed a timely notice of appeal on February 8, 2022. Appx20575. This Court has jurisdiction under 28 U.S.C. § 1295(a)(1).

STATEMENT OF THE ISSUES

This appeal concerns summary judgment of nonenablement of claims covering a small genus of antibodies. The issues presented are:

1. Whether the district court failed to hold Genentech to its burden to prove by clear-and-convincing evidence the facts underlying nonenablement and to construe the evidence in the light most favorable to Baxalta.

2. Whether the district court erred in treating the entire field of antibodies as an unpredictable art, despite evidence that skilled artisans could take an antibody that binds Factor IX/IXa and increases the procoagulant activity of Factor IXa and predictably engineer it into the different claimed isotypes and formats.

3. Whether the district court erred in treating the method of practicing the claims as trial-and-error, despite evidence that skilled artisans could consistently and predictably create and screen new antibodies that bind Factor IX/IXa and increase the procoagulant activity of Factor IXa, using a modification of the decades-old hybridoma-and-screening process held enabled in *Wands*.

4. Whether the district court applied the wrong legal standard for enablement by (a) requiring a patent claiming a novel quality to enable every possible measurement of that quality; (b) requiring enablement of antibodies outside the scope of the claims; and (c) focusing on development of the accused product, without evidence that its development followed the patent's teachings.

INTRODUCTION

The district court's grant of summary judgment turned on two factual errors: (1) "The field of antibodies is inherently unpredictable." Appx34; and (2) "The only way to practice the teachings of the patent is by trial-and-error; i.e., by screening tens of thousands, if not millions, of candidate antibodies to determine whether they satisfy the limitations of the asserted claims." Appx34. Neither is supported by the summary judgment record.

Admittedly, certain aspects of the field of antibodies are unpredictable. Skilled artisans cannot, for example, review the amino acid sequence of a variable region and know the antigen to which the antibody will bind or (generally) predict how changes to that sequence would affect it.

But other aspects of the field of antibodies are predictable. A skilled artisan in possession of an antibody that binds Factor IX/IXa and increases procoagulant activity could use well-known antibody engineering techniques to transform this antibody into any desired isotype or format. This is an aspect of the art where results are predictable—the record evidence shows that skilled artisans "could make predictable changes . . . to arrive at other types of antibodies" that also bind Factor IX/IXa and increase procoagulant activity. *AbbVie Deutschland GmbH & Co., KG v. Janssen Biotech, Inc.*, 759 F.3d 1285, 1301 (Fed. Cir. 2014).

This fact distinguishes *Amgen Inc. v. Sanofi*, 987 F.3d 1080 (Fed. Cir. 2021), in which the claims were not enabled because they claimed all possible combinations of sixteen different binding sites, but a skilled artisan could not predictably create the claimed combinations. *Id.* at 1087-88 & n.1. Here, in contrast, a skilled artisan could engineer antibodies into the full scope of claimed isotypes and formats—a process involving a predictable aspect of the field of antibodies.

Second, practicing the claims does not involve "trial-and-error" or randomly synthesizing antibodies while hoping to discover one that practices the claims. The specification describes a two-step process to practice the claims, first using hybridoma technology to generate a group of antibodies that bind Factor IX/IXa and second using a chromogenic assay (taught by the specification) to identify which of these antibodies increase the procoagulant activity of Factor IX/IXa. The inventors performed this process four times and created new antibodies that practiced the claims each time. Baxalta's expert explained there is no "maybe I'll find them." Appx19397. This process is reliable, not trial-and-error, and requires no undue experimentation, just like the hybridoma-screening process held enabled in *Wands*.

The reliability of this process distinguishes this case from *Idenix Pharmaceuticals LLC v. Gilead Sciences Inc.*, 941 F.3d 1149 (Fed. Cir. 2019), and *Wyeth & Cordis Corp. v. Abbott Laboratories*, 720 F.3d 1380 (Fed. Cir. 2013), which did not involve any equivalent to the predictable process taught by the '590

Patent, which has consistently generated new embodiments every time it has been followed. In *Idenix* and *Wyeth*, screening was necessary to **determine whether** any synthesized candidates practiced the claims. In the '590 Patent, screening is necessary only to **identify which** of the manufactured antibodies practice the claims.

The district court failed to heed this Court's direction to consider each case, even those involving genus claims, "based on the facts of that case and the evidence presented there." *Amgen*, 987 F.3d at 1088. On the record here, a reasonable jury could find facts establishing that the claims are enabled. The district court's grant of summary judgment was erroneous.

The alternative is to embrace what commentators have already suggested: that this Court has overruled *Wands sub silentio* and announced the "death of genus claims" as a matter of law. Dmitry Karshtedt et al., *The Death of the Genus Claim*, 35 HARV. J.L. & TECH. 1, 4 (2021). But if facts and the record continue to matter, a jury could find facts under which these claims satisfy the test for enablement.

STATEMENT OF THE CASE

Antibodies and the Coagulation Cascade

Antibodies are proteins that bind to "antigens." Ordinarily, antibodies are "antagonistic" and block or inhibit antigens. Appx16843. Less often, antibodies are "agonistic" and activate or promote antigens. Appx16843. This appeal involves special, agonistic antibodies invented by Baxalta scientists that are valuable in treating Hemophilia A.

Hemophilia A patients lack or have an insufficient amount of a protein known as "Factor VIII," which plays a crucial role in the coagulation cascade—the body's process for causing blood to coagulate or clot. Appx19022. In the coagulation cascade, Factor VIIIa (activated Factor VIII) and Factor IXa (activated Factor IX) activate Factor X, which leads to the formation of a clot. Appx19022; *see also* Appx141 at 1:17-19. If there is insufficient Factor VIII, then Factor X is not activated, the coagulation cascade ceases, and no clot forms. Appx19023.

The conventional treatment is "replacement therapy," artificially replacing the missing Factor VIII. Appx19023. For most patients, this works well, but approximately 25-30% develop "inhibitors," making replacement therapy less effective. Appx19022-19023.

Baxalta Discovers an Agonistic Factor IX Antibody

Baxalta scientist Dr. Friedrich Scheiflinger—a named inventor on the '590 Patent—discovered an alternative means of activating Factor X: agonistic antibodies that bind Factor IX or IXa and increase the procoagulant activity of Factor IXa. Appx141 at 2:29-33. These antibodies permit Factor IXa to activate Factor X in the absence of Factor VIII. Appx141 at 2:39-43.

The specification refers to these antibodies as achieving "Factor VIII-like activity." Appx142 at 3:9. But although the antibodies achieve a "Factor VIII-like" result, their mechanism is different. They do not duplicate Factor VIII.

The '590 Patent's specification teaches how to consistently and predictably generate these novel antibodies. First, it teaches how to produce antibodies that bind Factor IX or Factor IXa. Appx145 at 9:62-10:37. Using hybridoma technology dating back to 1975, the inventors immunized mice to generate anti-Factor IX/IXa antibody-secreting B-cells. Appx19135. The inventors then removed B-cells expressing the anti-Factor IX/IXa antibodies from the spleens of the mice and fused them to specific myeloma cells to create master clones, which were then subcloned to create a single hybridoma secreting a specific antibody. Appx19135. The '590 Patent's Example 1—entitled "Immunization of Immunocompetent Mice and Generation of Anti-FIX/IXa Antibody Secreting Hybridoma Cells"—details this process. Appx145 at 9:62-10:37.

Second, the '590 Patent teaches how to identify which of those antibodies exhibit "Factor VIII-like activity." Appx145-146 at 10:39-12:56. As Dr. Scheiflinger testified, this was uncharted territory, and the inventors "couldn't follow anyone else." Appx16891. Conventional chromogenic assays measured the activity of Factor VIII itself—the concept of "Factor VIII-like activity" did not exist—so the inventors needed to "do the base work" to determine how to measure Factor VIII-like activity. Appx16891.

The results of the inventors' efforts are described in the '590 Patent's Example 2, entitled "Assaying for FVIII-like Activity in Supernatants of Anti-FIX/FIXa Antibody Secreting Hybridoma Cells." Appx145-146 at 10:39-12:56. Example 2 describes modifications to a commercially available test-kit COATEST VIII:C/4® "[t]o allow high throughput screening" in a "downscaled [] microtiter plate format." Appx145 at 10:48. Example 2 details how long to incubate at specific temperatures, when to read absorbance, and how to compare the absorbency of the samples against the absorbency of a diluted Factor VIII reference standard using specific software. Appx145 at 10:43-67.

Developing the means to screen for Factor-VIII-like activity was, in the words of Dr. Scheiflinger, the "fundamental technical problem here." Appx16866. He explained that "you c[ould] have many ideas," but given the state of the art in 1998 and 1999, if you could not screen for them, "you were ending up nowhere."

Appx16866. A standard chromogenic assay, which "test[ed] for Factor VIII," Appx16887, could not be used to test for a Factor IXa activating antibody. Appx16890-16891. "[T]here is a fundamental difference" between the two. Appx16887. But by following the guidance of the '590 Patent, skilled artisans can easily screen for and identify these antibodies.

The reliability of the process described in the '590 Patent is crucial. **Every time** the inventors followed this process, they produced new antibodies that bind Factor IX or IXa and increase the procoagulant activity of Factor IXa. Appx145-146.

The inventors performed four hybridoma fusion experiments, numbered 193, 195, 196, and 198. Appx145 at 10:11-13. In each fusion experiment, after screening the hybridoma cells for Factor VIII-like activity (i.e., procoagulant activity), the inventors selected "several (5-15) master clones," which then "were identified and subjected to subcloning." Appx146 at 12:14-16; *see also* Appx146 at 11:16-18. The subcloning was used to make the lines homogenous. Appx146 at 12:16-17.

Figure 1 of the patent shows "[t]he results of a screening for FVIII-like activity" in certain cell lines derived from some of the fusion experiments. Appx146 at 11:1-4.

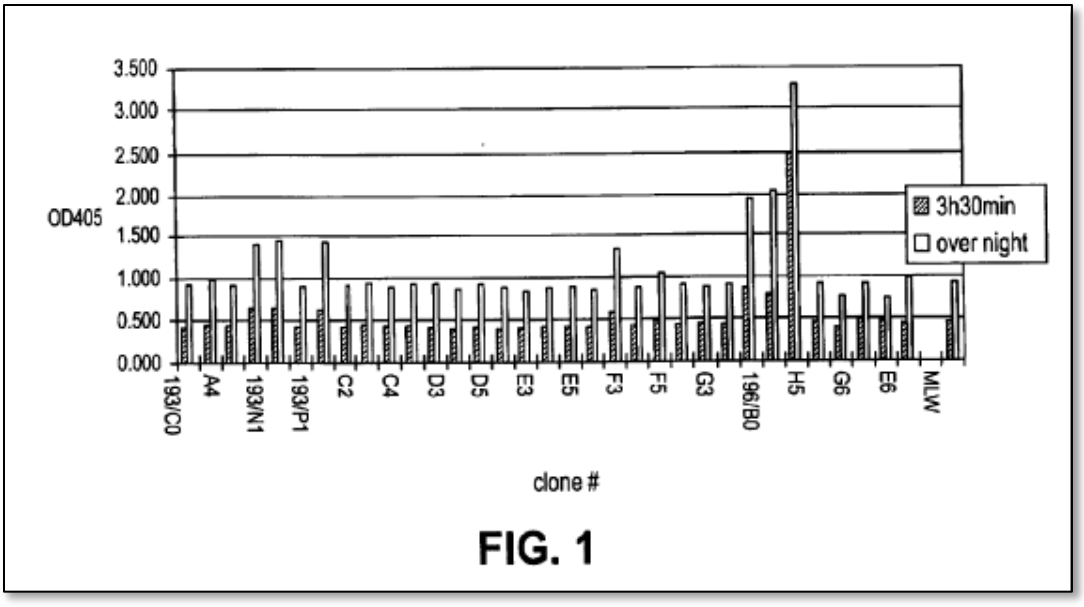


FIG. 1

Appx80. "MLW," the control, shows no Factor VIII-like activity. Appx146 at 11:14-15. Each other column is a cell line exhibiting Factor VIII-like activity.

Amino acid sequences are available for eleven of the antibodies made by the inventors (the "Disclosed Antibodies"). The inventors deposited several cell lines with the European Collection of Cell Cultures, where they remain accessible today. Appx19141-19142; Appx146 at 12:36-48. Others (in the form of scFvs) are disclosed in the '590 Patent by their amino acid sequences. Appx19141.

One of Baxalta's experts testified that by following the process taught by the '590 Patent, procoagulant antibodies were "surprisingly common." Appx19389. "[I]t was somewhat surprising that they could pick them up at that frequency." Appx19390. "[W]hen [the inventors] [went] through the methods . . . they g[o]t a number of monoclonal antibodies that c[a]me out of it." Appx19390. If a person

follows the process, they will find procoagulant antibodies. Appx19396-19397. There is no "maybe I'll find them"—you simply "have to go get them." Appx19397.

Given how easily (and predictably) this process generates new antibodies with the claimed functionality, a patent claiming only specific antibodies described by their amino acid sequences would be worthless. Because every time the process is followed, new antibodies—with distinct amino acid sequences—are made, a skilled artisan could simply follow the process taught by the '590 Patent and would, within weeks, Appx11434, possess a new antibody with the claimed properties.

For this reason, the '590 Patent claims the genus of antibodies with a specific functionality that can be produced using the disclosed process: "An isolated antibody or antibody fragment thereof that binds Factor IX or Factor IXa and increases the procoagulant activity of Factor IXa." Appx191, Claim 1.

Antibody Engineering

The hybridoma process described above produces "monoclonal monospecific" antibodies (antibodies produced by a single clone or cell line and that bind to a single antigen). The specification explains that after acquiring an antibody that binds Factor IX/IXa and increases procoagulant activity, a skilled artisan could

use well-known antibody engineering techniques to transform the antibody into any claimed isotype[1] or format.[2]

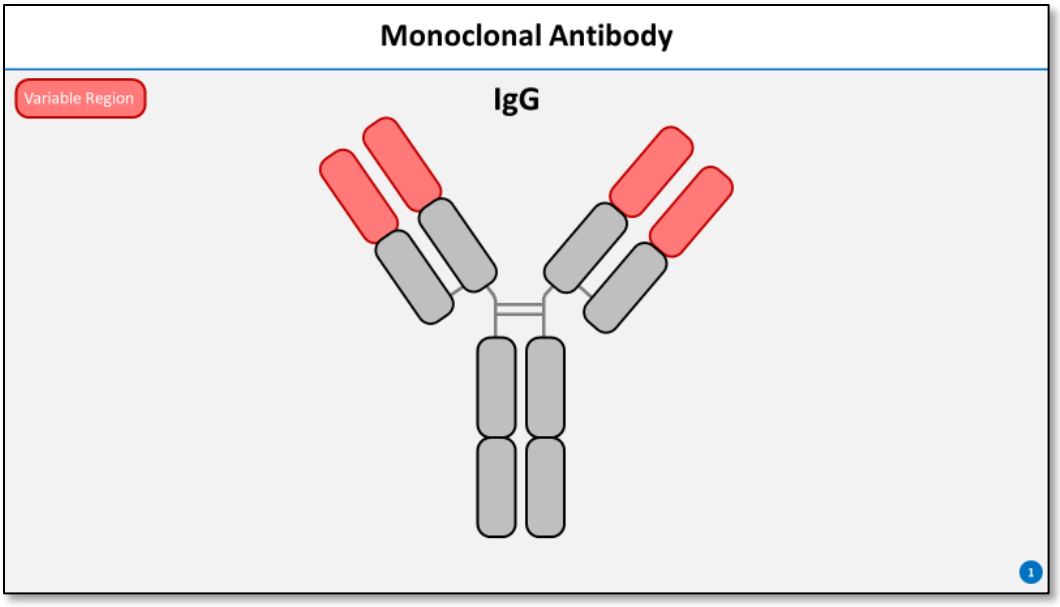
An antibody's "variable region"—specifically, the complementarity-determining regions ("CDRs")—is primarily responsible for its binding and functional properties, i.e., for whether the antibody binds Factor IX/IXa and exhibits procoagulant activity.[3] Appx19256. The rest of the antibody—its "constant region"—is "identical in all antibodies of the same isotype." Appx19256. "[T]he constant regions of IgG antibodies, for example, are identical but differ from the constant regions of IgA and IgM antibodies." Appx19256.

The following illustration shows an IgG antibody, with the variable regions in red and the constant regions in gray:

[1] Antibodies are grouped into five "classes" or "isotypes": IgM, IgD, IgG, IgE, and IgA. "Ig" stands for immunoglobulin, and the following letter signifies the specific class. Some isotypes also have subtypes.

[2] The '590 Patent claims a variety of antibodies, including monoclonal antibodies, chimeric antibodies, humanized antibodies, single chain antibodies (scFvs), bispecific antibodies, and diabodies, as well as dimers, oligomers, and multimers thereof. This brief refers to all of these as antibody "formats," even though some of them are (or might be) fragments. References to "antibodies" in the brief include "antibody fragments."

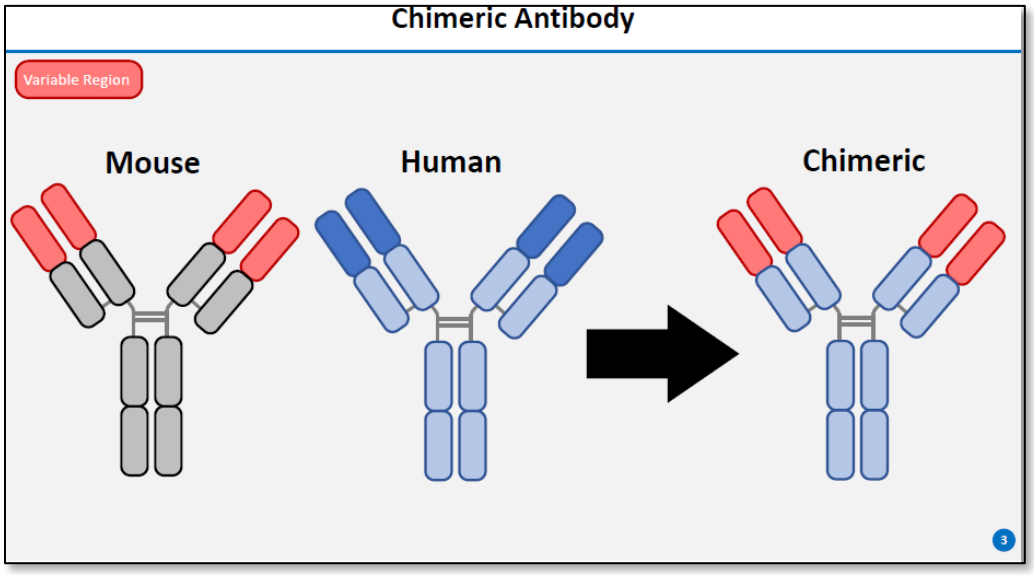
[3] Technically, an antibody has at least two "variable regions"—a heavy chain variable region (V_H) and light chain variable region (V_L), which are not identical. Some antibodies have multiple copies of each. This brief uses the singular "variable region" to refer to the combination of a V_H and V_L.



Appx20615. The two arms of the "Y" are identical. Because the antibody has two copies of the same variable region, it binds to a single antigen (i.e., it is "monospecific").

By following the process described in Example 1 and Example 2, a skilled artisan could create an IgM antibody that binds Factor IXa and increases procoagulant activity. The IgM antibody's variable regions could be combined with IgG constant regions to create an IgG antibody that exhibits the same claimed properties. Appx155-156 at 30:11-31:10 (detailing a class switch from IgM to IgG1); *see also* Appx143 at 6:41-48 (discussing "class switching"); Appx19212-19215. Similar class switching could be used to create IgG, IgM, IgA, and IgE antibodies that bind Factor IX/IXa and increase procoagulant activity. *See* Appx143 at 6:41-48 (noting that class switching is "known from the prior art").

Skilled artisans could also create "chimeric antibodies" that bind Factor IX/IXa and increase procoagulant activity. A chimeric antibody combines portions of antibodies from two species, such as "variable regions . . . of non-human origin" and human constant regions. Appx143-144 at 6:64-7:3. Combining the variable regions of a murine monoclonal antibody that binds to Factor IX/IXa and increases the procoagulant activity of Factor IXa with human IgG constant regions would create a chimeric IgG antibody that (predictably) binds to Factor IX/IXa and increases the procoagulant activity of Factor IXa:

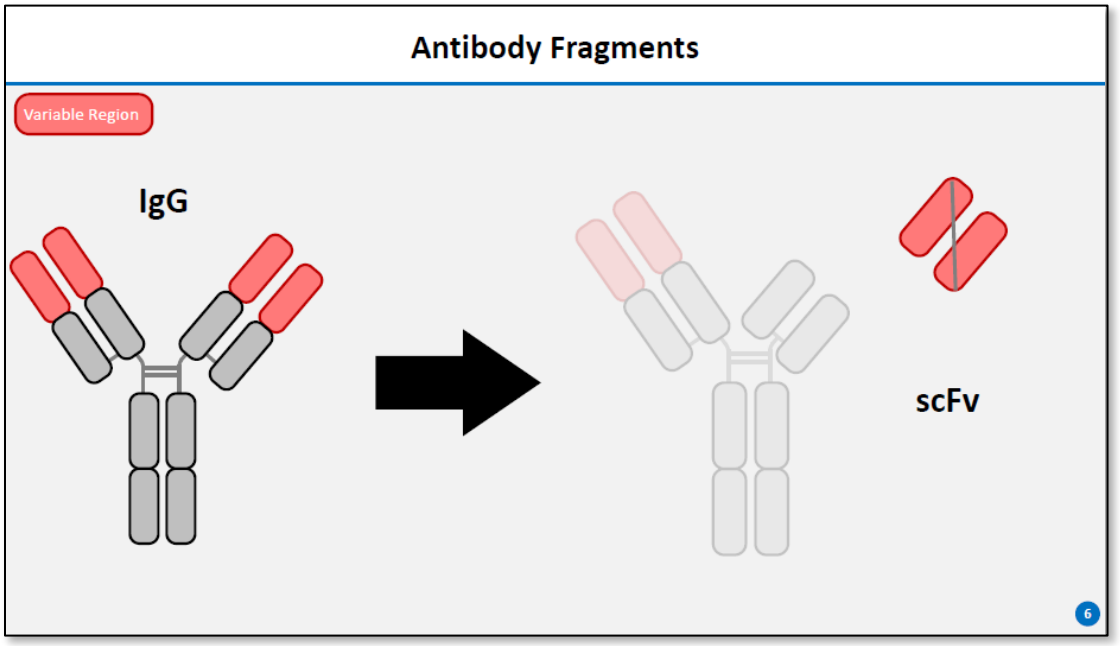


Appx20617; *see also* Appx19216-19218.

"Humanized antibod[ies]" include less than the entire non-human variable region, such as "complement[arity] determining regions (CDRs) from murine monoclonal antibodies" combined with human "framework regions." Appx143 at 6:49-53. Using "well-known technology" and "without undue experimentation,"

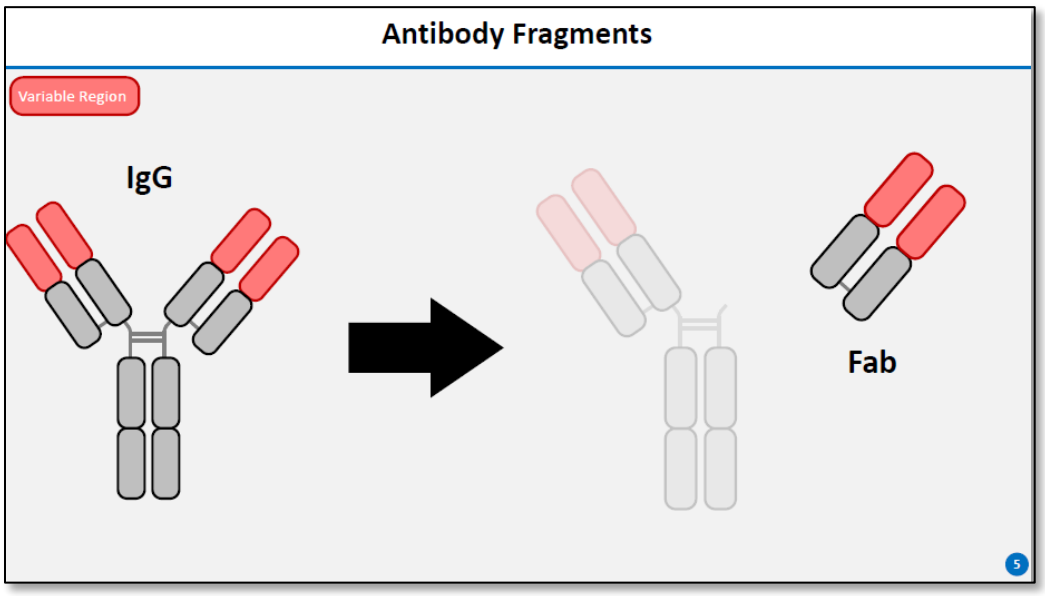
skilled artisans could combine the CDRs from a murine antibody that binds Factor IX/IXa and increases procoagulant activity with human framework regions and thus "make and use humanized antibodies that bind to Factor IX/Factor IXa and increase the procoagulant activity of Factor IXa." Appx19218-19220.

Skilled artisans could also engineer the variable region of an antibody that binds Factor IX/IXa and increases procoagulant activity into a single chain antibody (scFv), a type of antibody fragment that does not have constant regions. Skilled artisans could incorporate the variable region into "one single polypeptide chain," Appx144 at 7:9-10, by connecting its pieces using a "linker sequence," Appx144 at 7:21, 7:30-31.



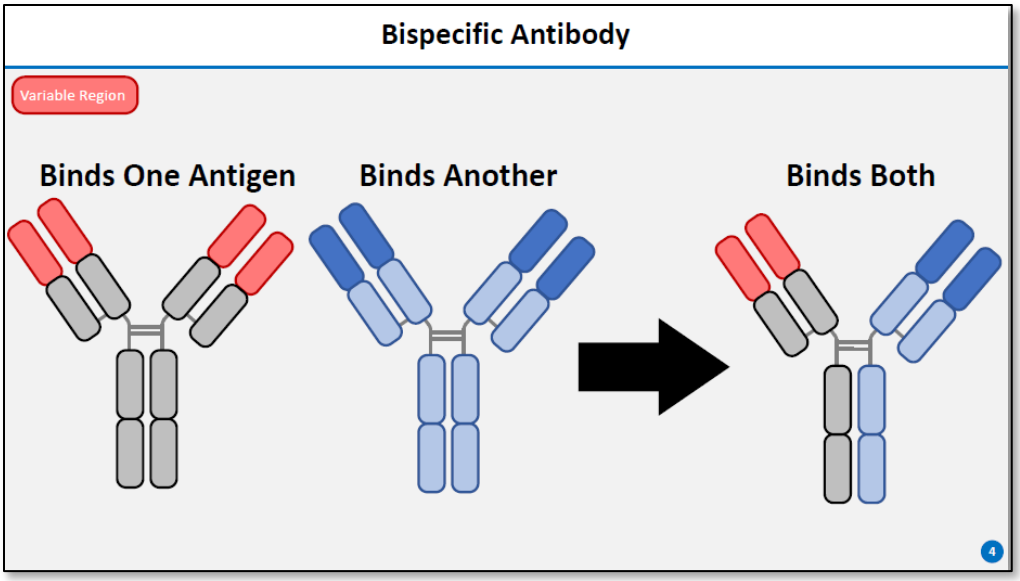
Appx20620. Creating scFvs was well known in the art, and the inventors made several. *See* Appx19191-19192 (discussing scFvs); Appx19186-19188 (discussing the examples in the specification).

Skilled artisans could also create "Fab" fragments—which have the variable region and portions of the constant regions—from antibodies that bind Factor IX/IXa and increase procoagulant activity.



Appx20619. "Fab fragments were well known and readily producible by a POSITA at the time of the invention." Appx19188; *see also* Appx19188-19191.

Bispecific antibodies have "two different binding specificities within one single molecule." Appx144 at 7:32-34. Skilled artisans "were readily familiar with bispecific antibodies" by 1999, and methods of producing them were well known. Appx19691-19693. To bind multiple antigens, a bispecific antibody needs different variable regions:

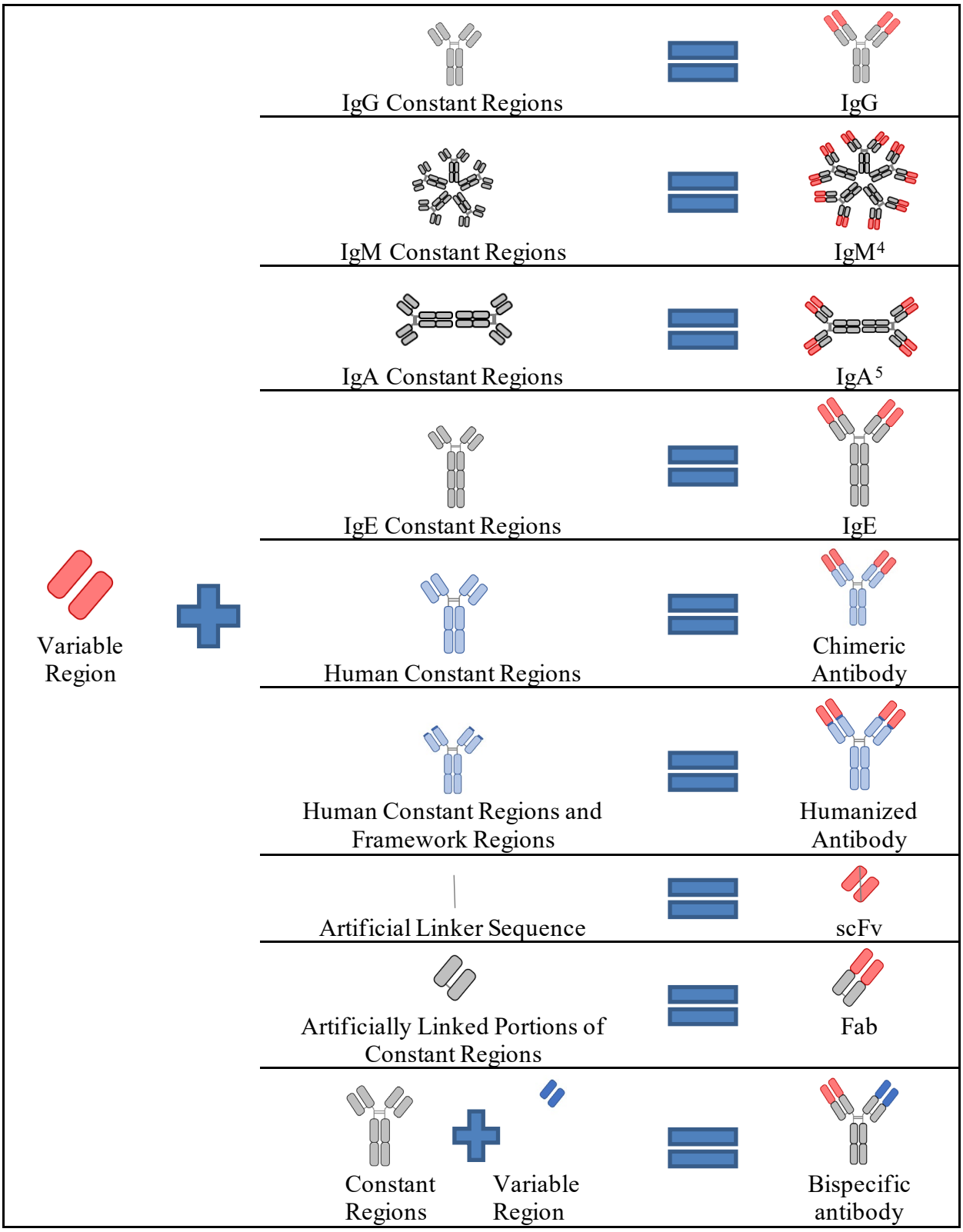


Appx20618. A skilled artisan could use the process taught by the '590 Patent to create a monoclonal antibody that binds Factor IX/IXa and increases the procoagulant activity of Factor IXa, replace one copy of its variable region with a variable region that binds a different antigen, and create a bispecific antibody that binds Factor IX/IXa and increases the procoagulant activity of Factor IXa. *See* Appx19677-19723 (Chang expert report discussing bispecific technology).

The same is true of diabodies and multimers. Appx19222-19226.

None of these techniques was discovered by the inventors of the '590 Patent. Skilled artisans were well aware of antibody engineering.

This chart illustrates that skilled artisans could create the claimed isotypes and formats by combining variable regions that bind Factor IX/IXa and increase the procoagulant activity of Factor IXa with constant regions or linkers (and, for bispecific antibodies, a second variable region):



[4] An IgM is a type of "multimer."
[5] An IgA is a type of "dimer."

Emicizumab and Genentech

Sometime between 1999 and mid-2000, Chugai Pharmaceutical Co., Ltd.[6] started to investigate the concept of bispecific antibodies to Factor IX/IXa and Factor X/Xa that could substitute for Factor VIII/VIIIa in hemophilia patients. Appx18248; Appx17314. Chugai began experimental work around May 2002. Appx18248.

Chugai scientists first generated hybridomas making monospecific antibodies against Factor IXa and Factor X. Appx18248-18249. They then combined these antibodies to create a bispecific antibody that binds Factor IXa with one arm and Factor X with another. Appx18249-18250. They then screened these antibodies for procoagulant activity, Appx18250, and humanized the lead antibody candidate. Appx18251.

After making a humanized bispecific antibody that bound Factor IXa and exhibited procoagulant activity, Chugai then "engaged in extraordinary antibody engineering to refine and further refine the candidate antibody." Appx16230. Chugai called the resulting antibody "emicizumab," which is now sold under the brand name "HEMLIBRA." Genentech received the rights to sell HEMLIBRA in the United States. Appx19021.

[6] Chugai and Genentech are both Roche subsidiaries. Appx394; Appx397.

In 2017, Baxalta sued Genentech and Chugai, alleging infringement of claims 1, 4, 17, and 19 of the '590 Patent.[7] Appx242.

Claim Construction and the Prior Appeal

In December 2018, the district court issued a claim construction order, construing, *inter alia*, "antibody" and "antibody fragment." Appx16160-16190. Based on the claim construction, the parties stipulated to non-infringement, Appx16195-16197, and the district court entered judgment, Appx16199-16202. Baxalta appealed.

This Court vacated the judgment of non-infringement and remanded, holding that the district court erred in construing "antibody" and "antibody fragment." *Baxalta Inc. v. Genentech, Inc.*, 972 F.3d 1341, 1343 (Fed. Cir. 2020). This Court construed "antibody" as "an immunoglobulin molecule having a specific amino acid sequence comprising two heavy chains (H chains) and two light chains (L chains)." *Id.* at 1349. And it construed "antibody fragment" as "a portion of an immunoglobulin molecule having a specific amino acid sequence comprising two heavy chains (H chains) and two light chains (L chains)." *Id.*

[7] Chugai was later voluntarily dismissed. Appx16033.

The District Court Grants Summary Judgment of Nonenablement

On remand, Genentech moved for summary judgment of invalidity for lack of written description and nonenablement.[8] Appx16215. Although written description and enablement are separate requirements, Genentech addressed them together. Appx16242; Appx16249-16252. In its enablement arguments, Genentech failed to discuss—or even cite—*In re Wands*, 858 F.2d 731 (Fed. Cir. 1988), or the *Wands* factors. Appx16217-16219.

In contrast, Baxalta's opposition cleanly separated written description and enablement and, in discussing enablement, addressed each of the *Wands* factors (including the factual disputes underlying these factors). Appx18861-18875. Baxalta supported its opposition with the testimony of two experts: Dr. Wayne Marasco and Dr. Sriram Krishnaswamy. Dr. Marasco holds a Ph.D. in Immunology and heads a research laboratory in cancer and infectious disease immunotherapy. Appx19121. He has worked in the antibody field since before 2000 and founded the National Foundation of Cancer Research Center for Therapeutic Antibody Engineering "to expand the use of human monoclonal antibodies in the treatment of cancer." Appx19122.

[8] Genentech also moved for summary judgment of non-infringement under a doctrine-of-equivalents theory and no willful infringement. Appx16211. The district court did not address written description or these other arguments and denied Genentech's motion on these grounds as moot. Appx76.

Dr. Krishnaswamy holds a Ph.D. in Biochemistry. Appx4344. At the University of Pennsylvania, he serves as Professor in the Department of Pediatrics, Professor (Secondary) in Systems Pharmacology and Translational Therapeutics, and Professor (Secondary) in the Department of Biochemistry and Biophysics as a Member of the Structural Biology Group. Appx4346. His primary research area concerns blood coagulation. Appx4346

After analyzing the *Wands* factors, Baxalta's experts concluded that skilled artisans would "not need to engage in undue experimentation to make and use the [claimed] antibodies or antibody fragments." Appx19530-19531 (Krishnaswamy); Appx19545; *see also* Appx19176 (Marasco: "[T]he '590 patent would have taught a POSITA how to make and use the claimed invention without undue experimentation.").[9]

Following a hearing, the district court granted Genentech's motion for summary judgment of invalidity for nonenablement. Appx30. The district court found that "Genentech has shown by clear and convincing evidence that the asserted claims of the '590 patent are not enabled." Appx32.

This appeal followed. Appx20575.

[9] In addition to these experts, Dr. Chien-Hsing Chang testified regarding bispecific antibodies. Appx19670-19723.

SUMMARY OF THE ARGUMENT

The district court erred in granting summary judgment of nonenablement because Genentech did not meet its burden of establishing invalidity by clear-and-convincing evidence in view of the summary judgment record of this case and the precedent of this Court.

The scope of the claims covers (1) isotypes and formats of antibodies with (2) variable regions that cause them to bind Factor IX/IXa and increase procoagulant activity. Practicing the claims thus involves two steps: (1) obtaining the variable region through the (a) hybridoma and (b) chromogenic assay process; and (2) engineering it into the desired isotype or format. The district court should have considered these two steps—which involve different technologies—separately.

Viewing the evidence in the light most favorable to Baxalta, skilled artisans would have been able to apply predictable antibody engineering techniques to generate antibodies in the claimed isotypes and formats that bind Factor IX/IXa and increase procoagulant activity. The inventors of the '590 Patent did not invent these engineering techniques and did not need to teach them in the specification.

The hybridoma–screening process taught by the '590 Patent to acquire new variable regions is reliable, requiring, at most, routine experimentation. Each time the inventors performed a hybridoma fusion experiment, they identified new

procoagulant antibodies. Appx20571-20572. Viewing the evidence in the light most favorable to Baxalta, the '590 Patent enables the claims' full scope.

The district court erroneously treated screening as undue experimentation as a matter of law, an approach inconsistent with the *Wands* factors and *Wands*'s holding. The district court made no attempt to harmonize its decision with this governing precedent.

The district court also erroneously imported an enablement requirement from *MagSil Corp. v. Hitachi Global Storage Technologies, Inc.*, 687 F.3d 1377 (Fed. Cir. 2012), which applies to claims for an improved measurement of a known quality. Here, the claims concern a new effect ("increasing procoagulant effect"), not any measurement of that effect. But the district court treated the '590 Patent claims as implicitly claiming any measured increase from zero to infinity and required enablement of every hypothetical measurement. This Court has rejected such a test, and few, if any, patents could survive it. *CFMT, Inc. v. Yieldup Int'l Corp.*, 349 F.3d 1333, 1338 (Fed. Cir. 2003).

The district court's enablement analysis also conflicts with its claim construction. The district court rejected Baxalta's explanation—calling it "convoluted"—that a bispecific antibody would be outside the scope of the claims if its procoagulant effect arose only from the non-Factor IX/IXa arm. Appx75. But the claim construction requires binding to Factor IX/IXa to "pla[y] a role in causing

an increase in procoagulant activity." Appx16186-16188. The district court erred by requiring Baxalta to enable unclaimed antibodies.

Finally, the district court erred by focusing on the accused product, emicizumab. Contrary to the district court's holding, an inventor is not required to discover and enable the best possible mode of practicing the claims. Emicizumab also cannot be relied on as post-priority evidence because Genentech contends that Chugai developed emicizumab using an approach "nowhere mentioned" in the '590 Patent, Appx19807, and emicizumab's development time was due, in part, to optimization steps to assist in its manufacturing. In any event, contrary to the district court's decision, an apples-to-apples comparison shows that emicizumab generates less activity (on a molar basis) than one of the Disclosed Antibodies.

ARGUMENT

I. Standard of Review

This Court reviews summary judgment *de novo*, applying the same standard as the district court. *Junker v. Med. Components, Inc.*, 25 F.4th 1027, 1032 (Fed. Cir. 2022). Summary judgment is appropriate only "if the movant shows that there is no genuine dispute as to any material fact and the movant is entitled to judgment as a matter of law." Fed. R. Civ. P. 56(a). A court must credit the non-movant's evidence and draw all reasonable inferences in the non-movant's favor. *Anderson v. Liberty Lobby, Inc.*, 477 U.S. 242, 255 (1986).

"[I]t is inappropriate to grant summary judgment in favor of a moving party who bears the burden of proof at trial unless a reasonable juror would be compelled to find its way on the facts needed to rule in its favor on the law." *El v. Se. Pa. Transp. Auth.*, 479 F.3d 232, 238 (3d Cir. 2007). "[I]f there is a chance that a reasonable factfinder would not accept a moving party's necessary propositions of fact, pre-trial judgment cannot be granted." *Id.* This standard is even higher where, as here, the burden is clear-and-convincing evidence. *Anderson*, 477 U.S. at 255.

Enablement is a question of law based on underlying factual findings. *Amgen*, 987 F.3d at 1084. "To prove that a claim is invalid for lack of enablement, a challenger must show by clear and convincing evidence that a person of ordinary skill in the art would not be able to practice the claimed invention without 'undue

experimentation.'" *Alcon Research Ltd. v. Barr Labs., Inc.*, 745 F.3d 1180, 1188 (Fed. Cir. 2014).[10]

A. The district court failed to hold Genentech to its burden and to construe the evidence in the light most favorable to Baxalta.

The district court failed to follow the summary judgment standard. Although the opinion recites the correct legal standard, its analysis does not address what a reasonable jury could (or must) find, refer to Genentech's burden of proof, or apply the clear-and-convincing-evidence standard.

At one point, the district court misstates the legal standard: "For the reasons described in detail below, the court **finds** that Genentech **has shown by clear and convincing evidence** that the asserted claims of the '590 patent are not enabled." Appx32 (emphasis added). Although the opinion later recites the correct standard—whether "no reasonable jury could find the full scope of the asserted claims of the '590 patent are enabled," Appx36—aside from a passing reference in the section discussing the dependent claims, Appx73, its analysis does not address what any reasonable juror must find that Genentech has proven by clear-and-convincing evidence.

[10] While acknowledging that they are presently foreclosed by this Court's precedent, Baxalta adopts the arguments raised in the pending petition for writ of certiorari in *Amgen v. Sanofi*, No. 21-757. Any change of the law of enablement by the Supreme Court in *Amgen* should apply in this case as well.

B. The district court failed to credit Baxalta's evidence.

The district court failed to view the evidence and draw all inferences in Baxalta's favor. These factual disputes are detailed below, but key examples include:

Guidance. The district court characterized the guidance as minimal and the process taught in the '590 Patent as "trial-and-error," Appx34, the equivalent of randomly generating antibodies and hoping to uncover one that would practice the claims.

To the contrary, Dr. Marasco testified that there is a "profoun[d]" difference between (i) following the process of the '590 Patent that reliably makes the claimed antibodies versus (ii) applying a random trial-and-error approach. Appx16451.

Nor did the district court credit the testimony of Dr. Krishnaswamy (and Dr. Scheiflinger) regarding the importance of the modifications to the chromogenic assay detailed in Example 2 of the specification, Appx19531-19532, Appx19539-19541, which solved the "fundamental technical problem" necessary to practice the claims. Appx16866.

Predictability of the Field. The district court found "[t]his area of art is inherently unpredictable," and "[t]he field of antibodies is itself unpredictable." Appx59; Appx34. But the district court cited no evidence for this proposition, Appx59, and it conflicts with the testimony of Baxalta's experts that the field is not

wholly unpredictable and a skilled artisan in possession of an antibody that binds Factor IX/IXa and increases procoagulant activity could, predictably, engineer that antibody into other isotypes and formats. Appx19207-19209; Appx19542-19543; Appx19722.

Number of Potential Candidates. The district court adopted the opinion of Genentech's expert Dr. Garcia that "the number of candidate antibodies or antibody fragments within the scope of claim 1 is high, 'encompassing millions of different structural formats, binding epitopes, binding affinities, and mechanisms of action.'" Appx49 (quoting Appx19337).

But Dr. Garcia never explained the basis for his assertion. Appx19337. He merely recited "millions" without providing any evidence or analysis in support. Appx19337. There is no reason that every reasonable juror would be required to credit this unsupported *ipse dixit*. *See TQ Delta, LLC v. CISCO Sys., Inc.*, 942 F.3d 1352, 1362 (Fed. Cir. 2019).

C. The district court's findings about the "true inventor" are irrelevant to the enablement inquiry.

Other findings both violate the summary judgment standard and are irrelevant. The last two pages of the opinion characterize "the situation" as Genentech doing "the hard work to invent a useful compound" and Baxalta as "some earlier inventor who may have conceived of such a theory or made the first step in research, but did not enable its ultimate production." Appx76; *see also* Appx75-76 (indicating that

Genentech is the "true inventor" and Baxalta is not). The district court announced that it "cannot allow Baxalta to provide a starting point for further research and then claim [Genentech's] solution to the problem." Appx76 (internal quotation marks omitted).

Enablement must be evaluated under the test prescribed by this Court—whether, under the *Wands* factors, practicing the claims requires undue experimentation—not under freestanding equitable considerations about the "true inventor" who "did the hard work," whether someone is trying to claim someone else's solution, and what the court "ca[n] allow." Appx76.

Even if they were relevant, these extraneous findings conflict with the summary judgment standard. There is no basis to conclude that every reasonable juror must find, by clear-and-convincing evidence, that Genentech was the "true inventor" who "did the hard work." The district court's views should not have affected its summary judgment ruling.

* * *

Summary judgment would be permissible only if Genentech presented "such clear and convincing evidence of facts underlying invalidity that no reasonable jury could find otherwise." *SRAM Corp. v. AD-II Eng'g, Inc.*, 465 F.3d 1351, 1357 (Fed. Cir. 2006). Genentech fell short. The parties' experts contested whether the

experimentation required to practice the '590 Patent is "undue," and the district court committed reversible error in resolving these disputes in Genentech's favor.

II. A Reasonable Jury Could Find Facts Under Which the '590 Patent Satisfies the Enablement Requirement.

Practicing the full scope of the claims involves two steps: First, obtaining a variable region that binds Factor IX/IXa and increases procoagulant activity. Second, engineering the variable region into the desired isotype or format.

Enablement, including an artisan's ability to extrapolate beyond disclosed embodiments, "depend[s] upon the predictability of the art[.]" *McRO, Inc. v. Bandai Namco Games Am. Inc.*, 959 F.3d 1091, 1102 (Fed. Cir. 2020). "If an invention pertains to an art where the results are predictable, . . . a broad claim can be enabled by disclosure of a single embodiment[.]" *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 1533 (Fed. Cir. 1987). In a predictable art, once a skilled artisan possesses a single enablement, "other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws." *In re Fisher*, 427 F.2d 833, 839 (C.C.P.A. 1970).

The district court overgeneralized in stating—without citation to any evidence—that "[t]he field of antibodies is inherently unpredictable." Appx34. True, some aspects are unpredictable: skilled artisans cannot predict how altering the amino acid sequence of an antibody's CDR (part of its variable region) will affect its binding. Appx17279-17280. But, taking the evidence in the light most favorable

to Baxalta, engineering an antibody that binds Factor IX/IXa and increases procoagulant activity into different isotypes and formats is a predictable art, in which a skilled artisan "could make predictable changes . . . to arrive at other types of antibodies" that also bind Factor IX/IXa and increase procoagulant activity. *AbbVie*, 759 F.3d at 1301.

Steps involving a predictable art and those involving an unpredictable art should be considered separately in evaluating enablement. The district court erred by characterizing the entire field as unpredictable and ignoring these differences.

A. Skilled artisans could, without undue experimentation, engineer an antibody that binds Factor IX/IXa and increases procoagulant activity into the various claimed isotypes and formats.

A patent must enable "the full scope of embodiments of the invention claimed." *McRO,* 959 F.3d at 1100. "When a patentee chooses to claim 'A or B,' . . . the specification must fully enable 'B' as well as 'A' when the differences between 'A' and 'B' substantially affect the practice of the invention." *Soitec, S.A. v. Silicon Genesis Corp.*, 81 F. App'x 734, 738 (Fed. Cir. 2003).

Here, the breadth of the claims—the "A or B"—is the Markush groups listed in Dependent Claims 3 and 20—IgG, IgM, IgA or IgE isotypes—and Dependent Claims 4 and 19—monoclonal antibodies, chimeric antibodies, humanized antibodies, single chain antibodies (scFvs), bispecific antibodies, and diabodies, as

well as dimers, oligomers, and multimers thereof.[11] A skilled artisan in possession of an antibody that binds Factor IX/IXa and increases procoagulant activity could, using known techniques and without undue experimentation, engineer that antibody into each of the claimed isotypes and formats.

By definition, antibody engineering involves making changes to an antibody to arrive at other embodiments with predictable performance characteristics. For example, combining the variable region of an antibody that binds Factor IX/IXa and increases procoagulant activity with the constant regions of a different isotype is a "predictable chang[e] . . . to arrive at [another] typ[e] of antibod[y]" that also binds Factor IX/IXa and increase procoagulant activity. *See AbbVie*, 759 F.3d at 1301.

In discussing antibody engineering, the district court found that there is "no assurance that, once the modifications are made, the antibody will retain the same functional qualities." Appx72. If by "no assurance," the district court meant "no guarantee," this statement is irrelevant to the enablement inquiry. What matters is that engineering the claimed antibodies is predictable and does not require undue experimentation, not that a skilled artisan is guaranteed to succeed. If by "no assurance," the district court meant "unlikely," this statement fails to view the summary judgment evidence in the light most favorable to Baxalta.

[11] Enablement of Dependent Claim 19 requires enablement only of humanized antibodies. Appx192.

The inventors of the '590 Patent did not invent these antibody engineering techniques and did not need to explain them: "A 'patent need not teach, and preferably omits, what is well known in the art.'" *McRO*, 959 F.3d at 1102 (quoting *Spectra-Physics,* 827 F.2d at 1534). A court must consider "the knowledge of an ordinarily skilled artisan to determine whether undue experimentation would have been required to practice the invention." *Bayer Healthcare LLC v. Baxalta Inc.*, 989 F.3d 964, 982 (Fed. Cir. 2021). The '590 Patent did not need to teach—and properly omitted—the details of these well-known antibody engineering techniques. For example, as Dr. Scheiflinger asked rhetorically, "Bispecifics have been known for a long time, why would we put anything here in this patent[?]" Appx16838.

In considering the full scope of the claims (at Appx71-Appx73), the district court did not specifically find that engineering any format or isotype would require undue experimentation.

a. The district court failed to credit Baxalta's evidence regarding humanized antibodies.

Viewing in the light most favorable to Baxalta, the evidence shows that the experimentation involved in making a humanized antibody that binds Factor IX/IXa and increases procoagulant activity would not be "undue." According to Dr. Marasco, a skilled artisan "would not need guidance or direction in the specification on the making of a humanized antibody using well-known techniques." Appx19218.

The district court appeared to assume that the confirmatory test required undue experimentation. *See* Appx72 (citing Appx57-58). But the portion of the expert report cited by the district court opines that "such confirmation testing would **not** constitute undue experimentation." Appx19211-19212 (cited by Appx58) (emphasis added).

Quoting Dr. Marasco's deposition, the district court stated that a humanized antibody "frequently does not have the same effectiveness as the original murine antibody." Appx57 (quoting Appx16432). But on the same page of the deposition, Dr. Marasco clarified that by performing "modifications within known parameters," a skilled artisan can "get [humanization] to work precisely if you want to absolutely transfer equal activity from murine to human." Appx16432.

The district court erred by failing to take the evidence regarding humanization in the light most favorable to Baxalta and holding that the specification provided inadequate guidance regarding humanized antibodies. Appx72.

b. The district court failed to credit Baxalta's evidence regarding bispecific antibodies.

The district court also erred in concluding that the specification provided insufficient guidance regarding bispecific antibodies. Appx72; *see also* Appx74-75. The '590 Patent directs a skilled artisan to *Bispecific Antibodies as Novel Bioconjugates* (1998), which discloses a variety of techniques for making them, Appx144 at 7:2-36, and Genentech's expert admitted that, "[p]rior to September 14,

1999, multiple approaches had been described in the literature to make bispecific antibodies." Appx19776.

Using standard antibody engineering techniques, a skilled artisan could, without undue experimentation, take an antibody that binds Factor IX/IXa and exhibits procoagulant activity, combine its variable region with constant regions and the variable regions of a different antibody, and produce a bispecific antibody that practices the claims. *See generally* Appx19671-19723. The claims do not require any particular target for the second binding arm or any functionality resulting from that second binding arm.

The district court failed to consider the evidence regarding bispecific antibodies in the light most favorable to Baxalta. Genentech's experts opined that to enable a bispecific antibody, the '590 Patent needed to teach the identity of the second binding arm. *E.g.*, Appx18215-18217; Appx18418-18426. Although not perfectly clear, Genentech's experts appear to assume that only certain binding arms could be used to create a bispecific antibody. But Baxalta's experts testified that a skilled artisan could practice the claims—generate a bispecific antibody that binds Factor IXa and has a procoagulant effect—and identify a binding specificity for the second binding arm without needing to be taught by the '590 Patent. Appx19691; Appx19694; Appx19711-19712; Appx19717-19718. In finding that skilled artisans

needed to be taught the target of the second binding arm, the district court failed to credit the testimony of Baxalta's experts.

* * *

Taking the evidence in the light most favorable to Baxalta, if skilled artisans possess an antibody with a variable region that binds Factor IXa and exhibits a procoagulant effect as claimed by the '590 Patent, they could use well-known and predictable antibody engineering techniques to transform this antibody into any of the claimed isotypes and formats.

2. The district court erred by comparing this case to *Amgen*, where the full scope could not be practiced without undue experimentation.

The district court erred in comparing this case to *Amgen Inc. v. Sanofi*, in which the claims recited a Markush group of binding sites: "wherein . . . the monoclonal antibody binds to at least one of the following residues: S153, I154, P155, R194, D238, A239, I369, S372, D374, C375, T377, C378, F379, V380, or S381." 987 F.3d at 1083. Unlike the isotypes and formats claimed in the '590 Patent, antibodies targeting these binding residues could not be generated using predictable techniques.

Because Amgen "ch[ose] to claim 'A or B,'" the specification had to enable antibodies binding to each combination of these binding residues without undue experimentation. *Soitec*, 81 F. App'x at 738. "[T]he specification must fully enable

'B' as well as 'A' when the differences between 'A' and 'B' substantially affect the practice of the invention." *Id.*

Amgen held that "[t]he binding limitation . . . require[s] undue experimentation." 987 F.3d at 1087. The broad "functional diversity" of the claims covered all sixteen binding residues.

> For example, there are three claimed residues to which not one disclosed example binds. And although the claims include antibodies that bind up to sixteen residues, none of Amgen's examples binds more than nine.

Id. & n.1. Whether "the full scope of the[se] functional limitations" could be achieved was "unpredictable." *Id.* This Court noted "the conspicuous absence of nonconclusory evidence that the full scope of the broad claims [i.e., all combinations of sixteen residues] can predictably be generated by the described methods." *Id.* at 1087-88. That is, although the *Amgen* patent described a method of generating antibodies, there was no evidence that this method could generate antibodies within the full scope (including antibodies that bound to all sixteen residues or antibodies that bound to S372, C375, or C378). *See* Brief of Defendants-Appellees in *Amgen Inc. v. Sanofi*, 2020 WL 3046260 (cited at 987 F.3d at 1087 n.1).[12]

[12] Nor was there any evidence that skilled artisans could select a particular binding residue and, without undue experimentation, generate a new embodiment that would bind to the selected residue.

As Appellee Sanofi explained in its brief, Amgen's patent defined the "range of claimed antibodies" "by where they bind to PCSK9." *Id.* at 51-52. Having defined the range in this manner, Amgen was required to—but failed to—enable the entire range of binding sites without undue experimentation. 987 F.3d at 1087.[13]

In contrast, the '590 Patent defines the claimed antibodies in terms of isotypes and formats. And the evidence, discussed above, shows that the '590 Patent enables this full range without undue experimentation. A skilled artisan in possession of an antibody that binds Factor IX/IXa and increases procoagulant activity could, applying a predictable art, engineer the antibody into any desired isotype or format. In contrast, a skilled artisan in possession of an antibody claimed by the *Amgen* patents that bound to certain residues could not make predictable changes to create an antibody that bound to different residues.

The district court rejected this argument by erroneously focusing on "working examples." *See* Appx66 n.14 ("[S]imilar to *Amgen*, there are various categories of

[13] Unlike obviousness and anticipation, a broader claim is not necessarily less likely to be enabled than a narrower claim. A "car powered by electricity" is broader than a "car powered by electricity generated by a cold-fusion reactor," but the broader claim might well be enabled where the narrower is not. *Ethicon Endo-Surgery, Inc. v. U.S. Surgical Corp.*, 93 F.3d 1572, 1582 n.7 (Fed. Cir. 1996) (holding that even though claim based on a specific location of a "lockout" was not "supported by [the] disclosure," a broader claim might still be viable: "If [the inventor] did not consider the precise location of the lockout to be an element of his invention, he was free to draft claim 24 broadly . . . to exclude the lockout's exact location as a limitation of the claimed invention.").

antibodies that are identified in the claims that are not represented by working examples."). But "working examples" is only one part of the enablement inquiry. The key in *Amgen* was the "absence of nonconclusory evidence that the full scope of the broad claims"—the full range of binding sites—"c[ould] predictably be generated by the described methods." 987 F.3d at 1087-88. Baxalta, in contrast, presented evidence that the full scope of its claims—the different antibodies and formats of antibodies that bind Factor IX/IXa and increase procoagulant activity—could predictably be generated by well-known antibody engineering techniques

B. Following the steps of the specification, skilled artisans can make new embodiments without undue experimentation.

Nor did Genentech establish that "the amount of effort needed to obtain embodiments outside the scope of the disclosed examples and guidance"—to obtain new antibodies (with new variable regions) that bind Factor IX/IXa and increase procoagulant activity—involved undue experimentation. *Amgen*, 987 F.3d at 1088.

1. The process taught by Example 1 and Example 2 consistently produces new antibodies within the scope of the claims.

A skilled artisan following the teachings of Example 1 and Example 2 will reliably and consistently—with, at most, routine experimentation—obtain new embodiments (i.e., new antibodies with new variable regions). The specification recites that the inventors conducted four hybridoma fusion experiments and, each time, successfully made new antibodies that bound Factor IX/IXa and exhibited

procoagulant activity. Appx20571; Appx146 at 11:1-12:56; *see also* Appx19396-19397.

2. This Court has held, in *Wands*, that routine screening of antibodies does not constitute undue experimentation.

"Enablement is not precluded by the necessity for some experimentation such as routine screening" of antibodies. *Amgen Inc. v. Sanofi*, 872 F.3d 1367, 1379 (Fed. Cir. 2017) (quoting *Wands*, at 736-37). The district court erroneously held that the routine screening required to practice the '590 Patent demonstrated nonenablement. Appx60-66.

The district court's decision cannot be harmonized with *Wands*, and the decision makes no attempt to do so. *Wands* involved hybridoma technology and commercial screening assays used to make and identify antibodies to a herpes virus. "This process entails immunizing animals, fusing lymphocytes from the immunized animals with myeloma cells to make hybridomas, cloning the hybridomas, and screening the antibodies produced by the hybridomas for the desired characteristics." 858 F.2d at 740.

This Court agreed that the "written specification fully enables the practice of their claimed invention because the monoclonal antibodies needed to perform the immunoassays can be made from readily available starting materials **using methods that are well known** in the monoclonal antibody art." *Id.* at 736 (emphasis added); *see also id*. at 740 ("The nature of monoclonal antibody technology is that it involves

screening hybridomas to determine which ones secrete antibody with desired characteristics. Practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody.").

Similarly, the '590 Patent teaches the skilled artisan both (1) how to make antibodies using conventional hybridoma technology and (2) how to screen to detect Factor VIII-like activity. As in *Wands*, skilled artisans carrying out the disclosed process successfully made new antibodies that satisfied the claim limitations. *See id.* ("Wands carried out this entire procedure three times, and was successful each time in making at least one antibody that satisfied all of the claim limitations."). As in *Wands*, skilled artisans know how to make antibodies and detect Factor VIII-like activity to produce the claimed antibodies. And as in *Wands*, the specification enables the claims. The district court did not distinguish *Wands* because there is no principled basis to do so.

The district court's focus on the number of antibodies that would need to be screened, Appx64, conflicts with *Wands*, which correctly recognized that "[i]n the monoclonal antibody art it appears that an 'experiment' is not simply the screening of a single hybridoma, but is rather the entire attempt to make a monoclonal antibody against a particular antigen." 858 F.3d at 740. Similarly, here, an "experiment" is not simply the screening of a single hybridoma or antibody, but is rather the process of making a monoclonal antibody against Factor IX/IXa that increases the

procoagulant activity of Factor IXa. *See* Appx146 at 12:14-15 (describing a "[hybridoma] fusion experiment"); Appx146 at 11:9-10 (describing a "screening experiment" involving a group of antibodies). The "experiment" required to make these antibodies entails no more experimentation than the enabled hybridoma-screening process in *Wands* itself.

3. Under the *Wands* factors, taken in the light most favorable to Baxalta, undue experimentation is not required to practice the claims.

When considered under the *Wands* factors and in the light most favorable to Baxalta, obtaining new embodiments from Example 1 and Example 2 of the '590 Patent does not require undue experimentation. In its motion for summary judgment, Genentech failed to cite *Wands* or address the *Wands* factors.

a. Quantity of experimentation necessary and amount of direction or guidance presented.

Dr. Marasco opined that "Examples 1 and 2 in the specification explain how to efficiently produce antibodies binding to Factor IX/Factor IXa and how to screen those antibodies for an increase in procoagulant activity of Factor IXa using conventional techniques with modifications specific to the claimed invention." Appx19176.

Following the '590 Patent's teachings, skilled artisans would "feel confident that they could . . . immunize an animal . . . with Factor IX/IXa and get antibodies out that have procoagulant return." Appx16450; *see also* Appx16421. "The

specification instructs a POSITA to use well-known, inexpensive, and efficient means of producing an antibody or fragment thereof that binds to Factor IX or Factor IXa and identifying those that increase the procoagulant activity of Factor IXa," and it would not require "substantial time and effort" to make and use the claimed invention. Appx19197-19198. And "a POSITA could conduct the chromogenic assay as disclosed in the specification with little time or effort." Appx19540-19541; Appx19543. As Dr. Marasco observed, "[I]t's the simplicity of it that's really elegant." Appx16451.

The specification's guidance "pave[s] the way" for a skilled artisan and eliminates the uncertainty in whether they would be found. Appx16451. The teachings do not remove the need for a skilled artisan to "go get them," but "it takes the unknown out of [it]." Appx16451. There would be no "trial and error." **Each time the steps of Examples 1 and 2, which require only routine experimentation, have been followed, new embodiments of the '590 Patent have been produced**.

Dr. Krishnaswamy similarly opined that screening "the antibodies or antibody fragments of claim 1 that bind to Factor IX or Factor IXa . . . for an increase in procoagulant activity of Factor IXa . . . would be routine with predictable results." Appx19530-19531. Example 2 offers "a detailed explanation of using chromogenic assays to measure for factor VIII-like activity in supernatants of hybridomas that secrete anti-Factor IX/Factor IXa antibodies." Appx19531-19532.

With the specification's guidance, "the POSITA need not experiment to determine any necessary modifications in the first instance." Appx19539; *see also* Appx19532; Appx19539-19540. In developing the chromogenic assay to detect Factor IXa-activating antibodies, the inventors solved the "fundamental technical problem" that made identification of these antibodies possible. Appx16866-16891. Without these discoveries, claimed antibodies could not be identified. Appx16890-16891. The district court erred by failing to credit the importance of this guidance.

And according to Dr. Krishnaswamy, only a focused number of potential antibodies would need to be screened. Appx19541-19542. In his view, a skilled artisan "would be able to implement the specification's direction, guidance, and working examples to make and use antibodies or antibody fragments thereof that increase procoagulant activity of Factor IXa using routine screening." Appx19531; *see also* Appx19531-19533; Appx19539-19540; Appx17340 (the patent "lays out a strategy" for "high throughput screening of antibodies that enhance the procoagulant activity of Factor IXa").

When properly taken in the light most favorable to Baxalta, the evidence shows that the quantity of experimentation required to practice the invention is low and the level of instruction is high.

b. The presence or absence of working examples.

By following the process of Example 1 and Example 2 of the specification, the inventors generated between 30 to 50 working examples of antibodies exhibiting procoagulant activity. *See* Appx146 at 12:14-15 (explaining that for each of the four fusion experiments, "several (5-15) master clones . . . were identified and subjected to subcloning"); Appx146 at 11:35-39 (explaining that subcloning was based on detecting "FVIII-like activity"). The investors also disclosed the amino acid sequences of eleven of these antibodies. Appx19147-19148. Dr. Marasco analyzed their variable regions—including their (i) amino acid sequences; (ii) CDRH3 lengths; (iii) binding domains; and (iv) canonical structures of CDRH1 and CDRH2—and determined that they demonstrated significant diversity. Appx19132. This was the same method of analyzing antibody diversity that was employed in *AbbVie*, 759 F.3d at 1285.

From his analysis, Dr. Marasco concluded that the Disclosed Antibodies' variable regions "reflect the structural diversity of the claimed genus." Appx19151. After performing a similar analysis on emicizumab, Dr. Marasco determined that some Disclosed Antibodies have a higher sequence identity (and thus are more structurally similar) to emicizumab than to other Disclosed Antibodies. Appx19163.

The eleven Disclosed Antibodies are representative of the diversity of variable regions within the (small) claimed genus. The presence of working examples favors enablement.

c. The nature of the invention and the breadth of the claims.

The '590 Patent is directed to antibodies that bind Factor IX or Factor IXa and increase the procoagulant activity of Factor IXa. These two functional requirements limit the number of possible embodiments. Appx19205-19206 (Dr. Marasco explaining that "claim 1's two functional requirements . . . would necessarily limit the number of possible embodiments from the outset"); Appx19337 (Dr. Garcia acknowledging that the "functional limitation—increases the procoagulant activity of Factor IXa—does decrease the number of antibodies and antibody fragments within the scope of the claims").

The parties agree that the breadth of the claims is "focused and small." Dr. Garcia opined that the number of antibodies "that would activate Factor IXa such that there is an increase in procoagulant activity, is a very, very minor sub-fraction." Appx19321-19322; *see also* Appx19134; Appx19206-19207. Dr. Marasco agreed: "[T]he genus is actually pretty small. I mean, I think it's focused and small[.]" Appx16417.

d. The state of the prior art, relative skill of those in the art, and predictability or unpredictability of the art.

Dr. Marasco opined that "the state of the prior art at the time of the invention, a POSITA's relative skill, and the art's predictability confirm that a POSITA would know how to make and use the invention without undue experimentation." Appx19207. He characterized the level of skill as "high," where a skilled artisan "would be readily familiar with the technology discussed in the patent and the technology known in the prior art," such as "technology and techniques for producing antibodies using hybridoma or phage technology, and using chromogenic or aPTT assays." Appx19207.

Dr. Krishnaswamy agreed that "[t]he relative skill of those in the art was significant." Appx19542-19543. "A person having this level of ordinary skill in the art would have a ready, working knowledge of using chromogenic and aPTT assays," and the inventors' modifications to the assays as described in the patent "would be easily implemented and understood by a POSITA without substantial time or effort." Appx19543. Thus, in Dr. Krishnaswamy's opinion, "undue experimentation would not be required." Appx19543.

Although the art is unpredictable in the sense that one cannot predict—solely from reviewing its amino acid structure—which antigen a variable region will bind or how all changes to a variable region will affect its functionality, the process detailed in the '590 Patent for producing these antibodies is reliable and predictable.

Every time this process has been followed, new antibodies within the scope of the claims have been created.

Considering the evidence in the light most favor to Baxalta under the *Wands* factors, the '590 Patent teaches how to create new (undisclosed) embodiments without undue experimentation.

e. The district court erred by treating this predictable, reliable process as trial and error.

Following the process in Examples 1 and 2 does not involve "trial-and-error," but instead, as Baxalta's evidence shows, predictably and reliably generates new antibodies that practice the claimed invention. There is no uncertainty about whether following the process will create a new antibody within the scope of the claims.

The district court analogized the '590 Patent's teachings to those of the patent in *Wyeth*. Appx65-66; Appx71. But in *Wyeth*, practicing the claims truly required trial and error in "an unpredictable and poorly understood field." 720 F.3d at 1386. There were "tens of thousands of candidate compounds" and no way to identify a new embodiment of the claimed genus short of randomly "synthesiz[ing] . . . each candidate compound" and then testing "to determine whether it has immunosuppressive and antirestenotic effects," *id.* at 1385, requiring "complicated and lengthy series of experiments in synthetic organic chemistry." *Id.* This approach provided no certainty regarding when—or whether—one would uncover a compound within the claimed genus.

Wyeth did not involve any equivalent to the predictable, reliable process taught by the '590 Patent, which has generated new embodiments (i.e., new antibodies with new variable regions) every time it has been followed.[14] This process begins with hybridoma technology, introduced in 1975, which generates monoclonal antibodies with predefined specificities. Appx19179.[15] Genentech's expert conceded that "people have been using that [technology] to generate antibodies for decades." Appx19179 n.132.

The specification describes how to use hybridoma technology to produce monoclonal antibodies that bind Factor IX or Factor IXa. Appx145 at 9:62-10:31. It is undisputed that hybridoma technology is reliable and deterministic.

And when applied to Factor IX/IXa, the hybridoma process does more than just produce antibodies that bind to Factor IX or Factor IXa. Every time the process has been followed, it has produced antibodies that increase the procoagulant effect of Factor IXa. Appx146 at 12:14-16.

[14] There is no suggestion that following this process generates only a subset of the variable regions that exhibit the claimed functionality. *Cf. Idenix*, 941 F.3d at 1162 (rejecting the argument that enablement could be based on "the subset of the claim that a POSA might practice").

[15] The title of Kohler & Milstein's seminal publication on hybridoma technology emphasizes the deterministic outcome of that process: "*Continuous cultures of fused cells secreting antibody of* ***predefined specificity***." Appx19179 n.132 (emphasis added).

Example 2 teaches how to modify a high-throughput screening process to identify these antibodies with procoagulant effect. Dr. Scheiflinger described the screening as "relatively easily done, very fast, not problematic at all." Appx16842. These steps generated new embodiments—new antibodies with new variable regions—every time they were followed. *See supra* pp. 9-11, 40-43.

In *Idenix*, there were "at least 'many, many thousands' of candidate compounds" and no way to determine which, if any, of these candidates practiced the claims, short of synthesizing them at random and screening them. 941 F.3d at 1157; *see also id.* ("enormous quantities of 2'-methyl-up nucleosides"); *id.* at 1158 ("billions of possible candidates").[16]

But the '590 Patent does not suggest synthesizing antibodies at random. It teaches a straightforward and predictable process to generate a small group of antibodies, which (every time it has been followed) includes novel antibodies within the claimed genus, and then a detailed screening method to identify which antibodies increase procoagulant activity.

The district court was wrong to call this process "a search for a needle in a haystack," Appx63, implying that success is uncertain and unlikely. If these

[16] The same is true of *Enzo Life Scis., Inc. v. Roche Molecular Sys., Inc.*, 928 F.3d 1340, 1349 (Fed. Cir. 2019), which involved somewhere between "tens of thousands" and "millions" of embodiments, with no way to identify them other than synthesizing at random and screening candidates.

antibodies are needles in a haystack, the '590 Patent teaches a process that easily locates a needle every time.

Put another way, in *Idenix* and *Wyeth*, screening was necessary to **determine whether** any synthesized candidates practiced the claims. In the '590 Patent, screening is necessary only to **identify which** antibodies practice the claims. The former was uncertain and nonenabled; the latter is reliable and enabled.

4. There is no evidence that the '590 Patent claims distinct categories of variable regions.

This Court also requires that a patent enable any distinct categories within the claims. For example, in *Automotive Technologies International, Inc. v. BMW of North America, Inc.*, the claims covered both electronic and mechanical sensors. 501 F.3d 1274, 1281 (Fed. Cir. 2007). To enable the full scope, the patent was required to enable both categories. *Id.* at 1285. "Disclosure of only mechanical side impact sensors does not permit one skilled in the art to make and use the invention as broadly as it was claimed, which includes electronic side impact sensors." *Id.* "Electronic side impact sensors are not just another known species of a genus consisting of sensors, but are a distinctly different sensor compared with the well-enabled mechanical side impact sensor that is fully discussed in the specification." *Id.* A patent must enable any "distinctly different" category of claimed embodiments. *Id.*

Similarly, in *AK Steel Corp. v. Sollac & Ugine*, the full scope of the claims covered both "Type 1" and "Type 2" aluminum coating, but the patent enabled only Type 2. 344 F.3d 1234, 1244 (Fed. Cir. 2003). And in *Liebel-Flarsheim Co. v. Medrad, Inc.*, the full scope of the claims included injector systems with and without pressure jackets, but the patent enabled only systems with pressure jackets. 481 F.3d 1371, 1380 (Fed. Cir. 2007).

There is no suggestion here that claimed antibodies include any "distinctly different" categories of variable regions. That is, there is no evidence that any variable regions within the antibodies covered by the claims were anything other than species within a single genus.

The district court focused on "therapeutic antibodies," but no evidence suggests that "therapeutic antibodies" are a "distinctly different" category of antibodies or anything other than species within the overall genus of antibodies that bind Factor IX/IXa and increase procoagulant activity, all of which are enabled through following the process taught in Examples 1 and 2 of the '590 Patent.

* * *

Genentech did not even argue the *Wands* factors in its motion for summary judgment. Taking the evidence in the light most favorable to Baxalta, generating a new antibody outside the disclosed examples in the '590 Patent does not require undue experimentation under *Wands*. And taking the evidence in the light most

favorable to Baxalta, a skilled artisan could use well-known (and predictable) antibody engineering techniques to transform an antibody that binds Factor IX/IXa and exhibits a procoagulant effect into any desired isotype or format that would exhibit the same functionality without undue experimentation under *Wands*.

III. The District Court Applied the Wrong Legal Standard for Enablement.

The district court also erred by equating "functional scope" with the "degree of procoagulant activity," requiring Baxalta to enable antibodies that fall outside the scope of the claims, and focusing on the accused product, emicizumab, in its enablement analysis.

A. The district court erred by equating "functional scope" with the "degree of procoagulant activity."

The inventors' contribution to the art was the invention of Factor IX/IXa antibodies with procoagulant activity, not antibodies with a greater degree of procoagulant activity than was previously known. The claims concern antibodies with a particular function, not a particular measurement of that function.

At Genentech's invitation, the district court erroneously treated the '590 Patent as claiming a range of function from zero to infinity and requiring enablement of every possible measurement of functionality. If this were the law, no patent

reciting a function could survive an enablement challenge, unless it claimed an explicit and narrow range of measurement.[17]

Unsurprisingly, no case supports the district court's extraordinary approach to enablement. *MagSil Corp. v. Hitachi Global Storage Technologies, Inc.*, on which the district court relies (Appx38-39), involved a marginal quantitative improvement: a change in resistance at room temperature of at least 10%. 687 F.3d at 1379. The patentees distinguished the prior art based on the increased degree of change, while expressly claiming a range that covered "resistive changes of . . . 1000%." *Id.* at 1382. In these circumstances, the *MagSil* patentees needed to enable the full range that they claimed.

"[W]hen a range is claimed, there must be reasonable enablement of the scope of the range." *AK Steel Corp.*, 344 F.3d at 1244. When an inventor's contribution to the art is a minor improvement in a known measurement, for example, a composition with a potency greater than 1.0, the inventor should not be permitted to "to dominate all such compositions having potencies greater than 1.0, including

[17] Consider the patent at issue in *FastShip, LLC v. United States*, which claimed a vessel with a waterjet that "increase[d] efficiency of the hull." 892 F.3d 1298, 1301 (Fed. Cir. 2018). Under the district court's reasoning, the *FastShip* patent implicitly claims the entire range of hull efficiency increase from zero to infinity and, to be valid, must enable every measurement of efficiency increase within that range, an impossible requirement.

future compositions having potencies far in excess of those obtainable from his teachings plus ordinary skill." *In re Fisher*, 427 F.2d 833, 839 (C.C.P.A. 1970).

But this principle has never previously been applied outside of circumstances where a patent expressly claims a quantitative range as its improvement over the prior art. *See, e.g.*, *Epistar Corp. v. Lowes Cos., Inc.*, No. CV-17-03219-JAK-KSX, 2020 WL 771096, at *5 (C.D. Cal. Feb. 11, 2020) ("wherein the light-emitting structure has a light output power of **more than** 4 mW at 20 mA current"); *Par Pharm., Inc. v. TWi Pharm., Inc.*, 120 F. Supp. 3d 468, 476 (D. Md. 2015), *aff'd*, 624 F. App'x 756 (Fed. Cir. 2015) ("claimed particle size range"); *TSI Inc. v. Azbil BioVigilant Inc.*, No. CV-12-00083-PHX-DGC, 2014 WL 1604860, at *4 (D. Ariz. Apr. 22, 2014) (involving "specific, numerical wavelengths" of lasers that were claimed but not enabled).

This is not a distinction based on the significance of the claimed improvements, as the district court suggested at Appx70. It is a difference based on the claims. Courts regularly distinguish *Magsil* on the basis that no range is claimed. A decision from the Western District of Wisconsin provides a thoughtful analysis of *MagSil* (and its predecessor, *In re Fisher*, 427 F.2d 833 (C.C.P.A. 1970)), explaining why these decisions apply only to patents that claim "a specific range." *ABS Glob., Inc. v. Inguran, LLC,* No. 14-CV-503-WMC, 2019 WL 4276647, at *1 (W.D. Wis. Sept. 10, 2019); *see also Advanced Fiber Techs. (AFT) Tr. v. J & L Fiber Servs.,*

Inc., No. 1:07-CV-1191, 2015 WL 1472015, at *17 (N.D.N.Y. Mar. 31, 2015) (distinguishing *MagSil* on the same basis).

Indeed, the district court's analysis conflicts with this Court's holding in *CFMT,* which involved claims for "a general system to improve the cleaning process for semiconductor wafers." 349 F.3d at 1338. This Court held that "if a patent claimed a system that achieved cleanliness up to a specified numerical particle-free range, then enablement would require disclosure of a method that enables one of ordinary skill to achieve that range without undue experimentation." *Id.* at 1338. But if the patent claimed an "improvement" without claiming a specified numerical range, then "the disclosure enables that invention by showing improvements in the overall system." *Id.*

The claims-at-issue in *CFMT* "state[d] no standard of cleaning," so this Court declined to require enablement of "removal of all contaminants." *Id.* Instead, "any meaningful 'cleaning' would satisfy the claimed goal of 'cleaning semiconductor wafers." *Id.* at 1340 (reversing district court invalidity conclusion "because the district court misapplied the law of enablement").

CFMT is directly on point. Here, the claims cover an antibody that increases the procoagulant activity of Factor IXa, without claiming the amount of that improvement. To paraphrase *CFMT*, "[a]ny meaningful [increase in procoagulant

activity] would satisfy the claims," and as in *CFMT*, enablement by increasing the procoagulant activity to a particular level (much less an infinite level) is not required.

B. The district court required Baxalta to enable antibodies that—under its claim construction—are outside the claims.

As a bispecific antibody, emicizumab binds to two antigens: Factor IXa and Factor X. The claims of the '590 Patent concern only the procoagulant effect from its Factor IXa arm. If the procoagulant activity arose only from emicizumab's Factor X arm, there would be no infringement.

The district court called this a "convoluted argument" that Baxalta "offered for the first time at the Summary Judgment hearing." Appx75. It accused Baxalta of "arguing that one bispecific antibody is perhaps not within the scope of the claims if its procoagulant activity results from its bispecific nature." Appx75.

As an initial matter, Baxalta was not making an argument; counsel was merely answering questions posed by the district court: "So there would be no infringement if the procoagulant activity came from the binding to Factor 10?" Appx20407.

And in any event, Baxalta's answers echoed the claim construction. The claim construction dispute was whether the increase in procoagulant activity must be caused entirely (Genentech's position) or partially (Baxalta's position) by the binding to Factor IX/IXa. Appx16187. In its *Markman* order, the district court held that although "binding to Factor IX/IXa [must] pla[y] a role in causing an increase

in procoagulant activity," it "need not be the **sole cause** of the increase." Appx16186-16188 (emphasis added).

Under the claim construction, Baxalta's explanation at the hearing was plainly correct. The district court erred by requiring enablement of antibodies that, under its own construction, fall outside the claims.

C. The district court's focus on enablement of the accused product, emicizumab, is misplaced.

The district court also erroneously found it "significant that the patent does not remotely enable the accused antibody, emicizumab." Appx73.

1. An inventor is not required to discover the optimal mode of practicing the claims.

The district court's reasoning resembles a heightened best mode requirement: Because (it concluded) a humanized bispecific antibody binding Factor X and Factor IXa (like emicizumab) is the best possible form of the antibody claimed in the '590 Patent, enablement required the inventors to identify and teach this form. But the statute required only that the specification "set forth the best mode **contemplated by the inventor** . . . of carrying out the invention." 35 U.S.C. § 112(a) (emphasis added). Thus, the question was whether, at the time of patent filing, the inventor subjectively "possessed a best mode of practicing the claimed invention," based on "the inventor's personal preferences." *Wellman, Inc. v. Eastman Chem. Co.*, 642

F.3d 1355, 1360 (Fed. Cir. 2011). There is no requirement for the inventor to discover and teach the best possible mode, much less as part of enablement.

Patent law recognizes that inventions can provide a springboard for future innovation. *See, e.g.*, *Hormone Research Found., Inc. v. Genentech, Inc.*, 904 F.2d 1558, 1568 (Fed. Cir. 1990) ("Merely because purer and more potent forms . . . might be produced using later-discovered technology does not necessarily mean that the '833 patent specification did not provide sufficient enabling disclosures[.]"); *U.S. Steel Corp. v. Phillips Petroleum Co.*, 865 F.2d 1247, 1251 (Fed. Cir. 1989) ("That the '851 claim may cover a later version of the claimed composition . . . relates to infringement, not to patentability."); *see also Gillette Co. v. Energizer Holdings, Inc.*, 405 F.3d 1367, 1371 (Fed. Cir. 2005) ("[T]he open language of claim 1 embraces technology that may add features to devices otherwise within the claim definition.").

The relevant enablement inquiry is whether the '590 Patent enabled a skilled artisan, without undue experimentation, to make a bispecific antibody binding Factor X and Factor IXa and exhibiting procoagulant activity. According to Baxalta's experts, it did. Appx19221-19222; Appx19670-19723. The district court erred by holding that the inventors were required to identify and disclose the (supposed) optimal binding specificity for the second arm.

2. The time required to develop emicizumab does not disprove enablement.

The district court also found it "[s]ignificant" that it "took Chugai over ten years of multi-phased experimentation and the screening of tens of thousands of candidate compounds to discover emicizumab." Appx74. But the time and effort it took **Chugai** to develop emicizumab cannot show nonenablement because emicizumab has additional unclaimed features.

"Enablement does not require an inventor to meet lofty standards for success in the commercial marketplace." *CFMT,* 349 F.3d at 1338. "Title 35 does not require that a patent disclosure enable one of ordinary skill in the art to make and use a perfected, commercially viable embodiment absent a claim limitation to that effect." *Id.* "[M]ost inventions require further development to achieve commercial success" and "additional inventive work does not alone show nonenablement." *Id.* at 1340.

Nor is there evidence that Chugai developed emicizumab by following the teachings of the '590 Patent. *See Johns Hopkins Univ. v. CellPro, Inc.*, 152 F.3d 1342, 1360-61 & n.29 (Fed. Cir. 1998) (rejecting post-priority evidence concerning the amount of experimentation required because the expert "did not copy the protocol described" in the specification). To the contrary, Genentech contends Chugai took an approach that was "nowhere mentioned" in the '590 Patent. Appx19807. That it took ten years for Chugai to develop emicizumab because it

included, *inter alia*, unclaimed optimization steps intended to assist in its manufacturing is irrelevant to enablement of the '590 Patents' claims.

3. Even if the relative effectiveness of emicizumab were relevant, the district court erred in considering the evidence.

The district court also erred in analyzing evidence of emicizumab's effectiveness. The district court found that emicizumab's procoagulant activity is "approximately 10%" of normal FVIII and far exceeds the procoagulant activity of "the 11 disclosed antibodies in the specification," which the district court found not to exceed "3.75%." Appx68-69.[18]

But the district court failed to consider Dr. Krishnaswamy's testimony that correctly analyzing emicizumab's activity requires considering the amount tested because emicizumab's activity is concentration-dependent, not absolute. *See* Appx19519 ("[I]t is not true . . . that emicizumab has 'about 10% of normal Factor VIII levels' generally."). The district court cited these portions of the report but either failed to credit them or misunderstood them. Appx74 & n.20 (citing Appx19519);[19] *see also* Appx51 (citing Appx19524-19525).

---

[18] *See also* Appx47 (noting "10%" for emicizumab); Appx51 (noting "3.75%" for the Disclosed Antibodies); Appx59 (stating that the Disclosed Antibodies "increase the procoagulant activity of Factor IXa by a small amount").

[19] Footnote 20 appears to reject Dr. Krishnaswamy's opinion, but it also appears to (correctly) acknowledge that the differences between testing emicizumab "at a diluted concentration level" and "at the treatment concentration level." Appx74 n.20. This confirms that emicizumab's effectiveness depends on its concentration.

The test measuring the activity of the 198/A1 involved **roughly 1/30th** the amount used in the test measuring activity of emicizumab at 10% normal Factor VIII. Taking the quantity into account (and thus making an apples-to-apples comparison on a molar basis), 198/A1 showed **greater** activity:

Test	Quantity	Activity	Activity on a Molar Basis
Emicizumab (Sampei/Uchida)	300nM	10% Factor VIII	$\frac{1}{3000}$ Factor VIII
198/A1 ('590 Patent, Fig. 25)	10.8nM	3.75% Factor VIII	$\frac{1}{288}$ Factor VIII
Emicizumab (Sampei)	30nM	1% Factor VIII	$\frac{1}{3000}$ Factor VIII

Appx19519-19520 (Factor VIII had "3000-fold differential" compared to emicizumab on a molar basis); Appx19525 (Factor VIII had "only a 288-fold differential" compared to 198/A1 on a molar basis).

Even if emicizumab's activity were relevant, taken in the light most favorable to Baxalta, emicizumab's procoagulant activity falls squarely within the range of procoagulant activity exhibited by antibodies described in the '590 Patent's specification.

* * *

And accounting for concentration, at least one Disclosed Antibody measures greater effectiveness. Appx19525.

The district court applied the wrong legal standard for enablement in numerous ways. It treated the hybridoma-and-screening process as undue experimentation as a matter of law; it misapplied *MagSil* by equating "functional scope" with the "degree of procoagulant activity"; and it placed undue emphasis on the accused product. These errors—along with the district court's failures to hold Genentech to its burden; credit Baxalta's evidence; and properly analyze the *Wands* factors—mandate reversal.

CONCLUSION & PRAYER FOR RELIEF

Using the decades-old hybridoma process and the screening taught in the '590 Patent, skilled artisans could, without undue experimentation, make and identify new variable regions that bind Factor IX/IXa and increase procoagulant activity of Factor IXa. They could then, without undue experimentation, engineer these variable regions into an antibody of any claimed isotype and format that binds Factor IX/IXa and increases the procoagulant activity of Factor IXa.

Summary judgment should be reversed and the case remanded.

Dated: June 10, 2022

Respectfully submitted,

MORGAN, LEWIS & BOCKIUS LLP

By: */s/ William R. Peterson*
William R. Peterson
1000 Louisiana St., Ste. 4000
Houston, TX 77002

Michael J. Abernathy
Christopher J. Betti
Maria E. Doukas
Karon N. Fowler
110 North Wacker Drive
Chicago, IL 60606

Julie S. Goldemberg
1701 Market Street
Philadelphia, PA 19103

Counsel for Appellants, Baxalta Inc. and Baxalta GmbH

ADDENDUM

ECF No.	Docket Text	Date Filed	Appx Page No(s).
573	[UNSEALED] MEMORANDUM OPINION. Signed by Judge Timothy Belcher Dyk on 1/13/2022. This order has been emailed to local counsel. (myr) (Entered: 01/13/2022)	1/13/2022	Appx30-76
574	ORDER granting 407 Genentech Inc.'s Motion for Summary Judgment. See the accompanying Memorandum Opinion for additional details. The parties shall meet and confer and propose any redactions to the Memorandum Opinion on or before January 18, 2022. Signed by Judge Timothy Belcher Dyk on 1/13/2022. (myr) (Entered: 01/13/2022)	1/13/2022	Appx77
—	United States Patent No. US 7,033,590 B1	04/25/2006	Appx78-192

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

BAXALTA INCORPORATED and BAXALTA GMBH,

Plaintiffs,

v.

GENENTECH, INC. and CHUGAI PHARMACEUTICAL CO., LTD.,

Defendants.

Civil Action No. 17-509-TBD

MEMORANDUM OPINION

Pending before the court is Genentech's motion for summary judgment. Genentech moves for summary judgment of 1) invalidity of claims 1–4, 19, and 20 of the '590 patent for lack of written description and enablement, 2) non-infringement under a doctrine-of-equivalents theory, and 3) no willful infringement. For the reasons stated below, the court GRANTS Genentech's motion for summary judgment of invalidity for lack of enablement and need not address Genentech's motion in all other respects.

I. PROCEDURAL HISTORY

On May 4, 2017, Baxalta Inc. and Baxalta GmbH (together, "Baxalta") brought suit against Genentech, Inc. and Chugai Pharmaceutical Co., Ltd., alleging infringement of U.S. Patent No. 7,033,590 ("the '590 patent") by the manufacture, use, sale, offer to sell, and importation of an antibody used to treat hemophilia A and known as emicizumab or ACE910, marketed under the brand name Hemlibra. Compl., ECF No. 1, ¶¶ 37–51. Chugai was subsequently dismissed from

the case. [1] Genentech answered on June 30, denying Baxalta's allegations and counterclaiming for declaratory judgment of noninfringement and invalidity on grounds of lack of enablement and written description support. Answer & Countercl., ECF No. 9, ¶¶ 37–51, 120–49.

On December 14, 2017, Baxalta moved for a preliminary injunction against Genentech. *See* Mot. for Prelim. Inj., ECF No. 41. On August 7, 2018, the court denied Baxalta's motion, finding that it had not proven a likelihood of success with respect to infringement and invalidity, and that even if it had, "given the ample evidence of medical need, the public interest weigh[ed] strongly against issuing a preliminary injunction since Hemlibra has unique medical benefits not available from Baxalta's competing products." Prelim. Inj. Order, ECF No. 262, at 24; *id.* at 28–29.

On December 3, 2018, following a Markman hearing, the court issued a claim construction decision in which it construed the term "antibody" to exclude bispecific antibodies. *See* Claim Construction Order, ECF No. 330, at 22–23. Thereafter, the parties stipulated to non-infringement of the asserted claims under the court's claim construction. *See* Stipulations, ECF Nos. 331–332. The court entered judgment in Genentech's favor on February 1, 2019. *See* Stip. & Final J., ECF No. 337. Baxalta appealed, and on August 27, 2020, the Federal Circuit issued a decision rejecting this court's construction of the terms "antibody" and "antibody fragment," determining that the term antibody included bispecific antibodies, and vacating the judgment of non-infringement and remanding for further proceedings. *See Baxalta Inc. v. Genentech, Inc.*, 972 F.3d 1341, 1343, 1349 (Fed. Cir. 2020) (construing antibody to mean "an immunoglobulin molecule having a

[1] Chugai is a Japanese company that invented and manufactures the accused product, Hemlibra, in Japan. *See, e.g.*, Yamaguchi Decl., ECF No. 20, ¶¶ 2, 5. Hemlibra is shipped to the United States where it is sold by Genentech. *See id.* ¶¶ 7, 10. The parties stipulated to the dismissal of Chugai as a defendant in this case in June 2018. *See* Stip. & Prop. Order, ECF No. 220; July 2, 2018, Min. Entry.

specific amino acid sequence comprising two heavy chains (H chains) and two light chains (L chains)" and "antibody fragment" to mean "a portion of an antibody"). Upon remand, the case proceeded with fact and expert discovery.

On September 3, 2021, Genentech filed a motion for summary judgment of 1) invalidity of claims 1–4, 19, and 20 of the '590 patent for lack of written description and enablement, 2) non-infringement under a doctrine-of-equivalents theory, and 3) no willful infringement. *See* Opening Br. in Supp. of Genentech's Mot. for Summ. J., ECF No. 416 (Def.'s Mot.), at 1, 15–16. Baxalta thereafter filed its opposition to Genentech's motion, *see* Pl.'s Opp'n to Def.'s Mot. for Summ. J., ECF No. 424 (Pl.'s Opp'n), and Genentech filed its reply on October 15, 2021, *see* Reply Br. in Supp. of Genentech, Inc's Mot. for Summ. J., ECF No. 425. The parties have submitted expert declarations and exhibits, as well as a Joint Stipulation of Fact. *See* Joint Stip. of Fact Regarding Hybridoma Tech. & the Number of Anti-Factor IX/IXa Antibodies Disclosed in the '590 Patent, ECF No. 437 (Joint Stip.). The court heard oral argument on the motion on November 19, 2021. *See* Nov. 22, 2021, Min. Entry.

II. SUMMARY OF DECISION

For the reasons described in detail below, the court finds that Genentech has shown by clear and convincing evidence that the asserted claims of the '590 patent are not enabled. There are millions of candidate antibodies within the genus and a dearth of working examples of those that satisfy the claim limitations. There are only eleven working examples disclosed in the patent. The examples are all murine, monospecific antibodies of the IgG and IgM isotypes, or fragments thereof. The genus of independent claim 1 is functionally and structurally broad. And in many respects there are no examples in the specification for the covered classes of antibodies. For example:

1. Claim 1 covers an antibody that increases the procoagulant activity of Factor IXa by an amount ranging from barely perceptible to an amount capable of use in "a preparation for the treatment of blood coagulation disorders which has particular advantages for factor VIII inhibitor patients." '590 patent, col. 2, ll. 25–28. Claim 1 thus covers an antibody or antibody fragment that increases the procoagulant activity of Factor IXa in the presence of Factor VIII inhibitors.[2] There is no working example of an antibody that increases the procoagulant activity of Factor IXa by more than a marginal amount in the presence of Factor VIII inhibitors. And for the non-inhibitor population, there is no working example of an antibody that increases the procoagulant activity of Factor IXa by an amount capable of moving a patient with a severe hemophilia A condition (comprising over 60% of hemophilia A patients) to a mild condition. Baxalta's expert concedes that the patent's assertions that antibodies of the invention have therapeutic utility is merely "aspirational."
2. Claim 1 covers humanized and chimeric antibodies. There are no working examples of humanized or chimeric antibodies disclosed in the specification.
3. Claim 1 covers bispecific antibodies such as the accused product emicizumab. There are no working examples of bispecific antibodies disclosed in the specification.
4. Claim 1 covers antibodies of the IgE isotype. There are no working examples of IgE antibodies disclosed in the specification.

[2] As discussed below, an "inhibitor patient" is someone who has developed an immune response to traditional Factor VIII replacement therapies.

5. Claim 1 covers antibodies of the IgA isotype. There are no working examples of IgA antibodies disclosed in the specification.

6. Claim 1 covers antibodies of the IgD isotype. There are no working examples of IgD antibodies disclosed in the specification.

7. Claim 1 covers diabodies and dimers, oligomers, and multimers of the claimed antibodies. There are no working examples of diabodies or dimers, oligomers, or multimers of antibodies in the specification.

The specification also provides no guidance as to how to identify which antibodies will satisfy the claim limitations, nor does it describe what structural or other features of the disclosed antibodies cause them to bind to Factor IX/IXa or to increase the procoagulant activity of Factor IXa. The field of antibodies is inherently unpredictable. The only way to practice the teachings of the patent is by trial-and-error; *i.e.*, by screening tens of thousands, if not millions, of candidate antibodies to determine whether they satisfy the limitations of the asserted claims.

The same deficiencies exist as to dependent claims 3–4, 19, and 20, which include the same functional limitations as claim 1 but also specify structural limitations.

This is not adequate enablement under Federal Circuit precedent, including *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988), and the cases that have followed, because it requires undue experimentation to practice the full scope of what is claimed.

III. LEGAL STANDARD

A. Summary Judgment

Under Rule 56(a) of the Federal Rules of Civil Procedure, "[t]he court shall grant summary judgment if the movant shows that there is no genuine dispute as to any material fact and the movant is entitled to judgment as a matter of law." The moving party bears the burden of

demonstrating the absence of a genuine issue of material fact. *See Celotex Corp. v. Catrett*, 477 U.S. 317, 323 (1986). If the moving party has carried its burden, the nonmovant must then "come forward with 'specific facts showing that there is a *genuine issue for trial*.'" *Matsushita Elec. Indus. Co. v. Zenith Radio Corp.*, 475 U.S. 574, 587 (1986) (quoting Fed. R. Civ. P. 56(e)). The court "must draw all reasonable inferences in favor of the nonmoving party, and it may not make credibility determinations or weigh the evidence." *Reeves v. Sanderson Plumbing Prods., Inc.*, 530 U.S. 133, 150 (2000).

B. 35 U.S.C. § 112

One of the statutory conditions for patentability under the Patent Act is adequate disclosure of the invention. Section 112 provides, in pertinent part, that:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

35 U.S.C. § 112. Section 112 imposes two separate requirements. The first is the written description requirement, found in the first sentence of Section 112, which requires that the specification contain an adequate "written description of the invention." 35 U.S.C. § 112; *see also Ariad Pharms., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1346 (Fed. Cir. 2010) (en banc) ("[A] separate requirement to describe one's invention is basic to patent law . . . It is part of the *quid pro quo* of a patent; one describes an invention, and, if the law's other requirements are met, one obtains a patent."). The inquiry into written description is a question of fact but it is "amenable to summary judgment in cases where no reasonable fact finder could return a verdict for the non-

moving party." *Bos. Sci. Corp. v. Johnson & Johnson*, 647 F.3d 1353, 1361 (Fed. Cir. 2011) (internal quotation marks and citation omitted).

The second requirement is enablement. "Whether a claim satisfies the enablement requirement of 35 U.S.C. § 112 is a question of law." *Amgen Inc. v. Sanofi, Aventisub LLC*, 987 F.3d 1080, 1084 (Fed. Cir. 2021). An enabling disclosure is the "*quid pro quo* of the right to exclude." *J.E.M. Ag Supply, Inc. v. Pioneer Hi–Bred Intern., Inc.*, 534 U.S. 124, 142 (2001). To be enabling, "the specification of a patent must teach those skilled in the art how to make and use the full scope of the claimed invention without undue experimentation." *Genentech, Inc. v. Novo Nordisk A/S*, 108 F.3d 1361, 1365 (Fed. Cir. 1997) (quoting *In re Wright*, 999 F.2d 1557, 1561 (Fed. Cir. 1993)); *see In re Fisher*, 427 F.2d 833, 839 (C.C.P.A. 1970) ("[T]he scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art."). Although it is not necessary to disclose every species within a genus, *see In re Angstadt*, 537 F.2d 498, 502–03 (C.C.P.A. 1976), "there must be sufficient disclosure, either through illustrative examples or terminology, to teach those of ordinary skill how to make and how to use the invention as broadly as it is claimed," *In re Vaeck*, 947 F.2d 488, 496 (Fed. Cir. 1991).

To be valid, a patent must satisfy both the written description and enablement requirements. *See Ariad*, 598 F.3d at 1351. Because, for the reasons discussed below, the court finds that no reasonable jury could find the full scope of the asserted claims of the '590 patent are enabled, it need not separately address written description, though the claims may also be invalid for lack of written description support.

IV. FACTUAL BACKGROUND

The '590 patent is directed to an antibody or antibody derivative that binds to a protein important for blood coagulation known as Factor IX (or Factor IXa) and increases the procoagulant activity of Factor IXa, for use in treatment of hemophilia A patients, particularly those who have developed Factor VIII inhibitors. '590 patent, col. 2, ll. 25–33. Asserted here are independent claim 1 and dependent claims 2–4, 19 and 20, which recite:

> 1. An isolated antibody or antibody fragment thereof that binds Factor IX or Factor IXa and increases the procoagulant activity of Factor IXa.
>
> 2. The antibody or antibody fragment according to claim 1 that increases the procoagulant activity of Factor IXa in the presence of Factor VIII inhibitors.
>
> 3. The antibody or antibody fragment according to claim 1 wherein the antibody is an IgG, IgM, IgA or IgE antibody.
>
> 4. The antibody or antibody fragment according to claim 1, wherein said antibody or antibody fragment is selected from the group consisting of a monoclonal antibody, a chimeric antibody, a humanized antibody, a single chain antibody, a bispecific antibody, a diabody, and di-, oligo- or multimers thereof.
>
> 19. The antibody or antibody fragment according to claim 4, wherein the antibody is a humanized antibody.
>
> 20. The antibody or antibody fragment according to claim 2, wherein the antibody is selected from the group consisting of an IgG, IgM, IgA, or IgE antibody.

Some background on antibodies and hemophilia A as well as its prior-art treatment based on the parties' agreed-upon views is necessary.

A. Hemophilia A and Its Prior-Art Treatment

The body stops bleeding by relying on blood coagulation, also known as clotting, which is accomplished through a cascade of reactions between proteins. *See* Sheehan Decl., ECF No. 411,

Ex. 1, Opening Rpt. ¶¶ 23–24 (Sheehan Rpt.); Pl.'s Opp'n, Ex. 8, ECF No. 424-9, Krishnaswamy Rebuttal Rpt. ¶ 25 (Krishnaswamy Rpt.). The individual coagulation proteins are referred to as coagulation "Factors," with respective assigned Roman numerals (*e.g.*, Factor VIII and Factor IX). Sheehan Rpt. ¶ 23; Krishnaswamy Rpt. ¶ 25. These Factors normally circulate in the blood in inactive forms until triggered by a vascular injury, which causes a coagulation cascade. *See* Sheehan Rpt. ¶ 23. Factors in their activated form are identified with an appended "a" (*e.g.*, Factor IXa). *See id.*; Opp'n Br., Ex. 2, Malackowski Opening Rpt. (Malackowski Rpt.), at 20. The relevant steps in the clotting cascade for present purposes are the coming together of Factor VIIIa and Factor IXa. *See* Sheehan Rpt. ¶ 25; Krishnaswamy Rpt. ¶ 26. In a healthy person, activated Factor VIII (Factor VIIIa) "complexes with" activated Factor IX (Factor IXa) and Factor X, causing Factors IXa to activate Factor X to Factor Xa, which is essential for clot formation. *See* Sheehan Rpt. ¶ 25; Krishnaswamy Rpt. ¶ 26.

Hemophilia A is a genetic disorder in which patients lack sufficient functional Factor VIII. Young Decl., ECF No. 414, Rebuttal Report ¶ 14 (Young Decl.); Krishnaswamy Rpt. ¶ 27. This amounts to a roadblock in the clotting cascade, and hemophilia A patients therefore suffer from a reduced ability to form quick and effective blood clots. Without Factor VIII, and without treatment, hemophilia A patients are at risk of bleeding episodes not only from external trauma, but internally into joints and other spaces in the body. Young Decl. ¶ 14. Hemophilia A can be classified as mild, moderate, or severe, depending on the relative level of Factor VIII present. Sheehan Rpt. ¶¶ 31–32; Malackowski Rpt. at 21. There are approximately 23,000–25,000 males with hemophilia A living in the United States. Young Decl. ¶ 16. About half of them have been diagnosed with a severe form of the disorder. *Id.* Females are less likely to have severe

hemophilia A because the genetic mutation associated with hemophilia A is "X-linked recessive." Young Decl. ¶ 15.

Historically, the only treatment for hemophilia A patients was infusion (intravenous) with a Factor VIII replacement, either as needed when bleeding episodes occur (on-demand) or in a preventative matter (prophylaxis). *Id.* ¶ 17; Krishnaswamy Rpt. ¶ 28. The problem with that treatment, however, was that 25–30% of patients with severe hemophilia who were treated with Factor VIII replacement therapies developed an immune response to Factor VIII. Young Decl. ¶ 22; Malackowski Rpt. at 21. This immune response is known as an "inhibitor," and the patients exhibiting this response are known as the "inhibitor population." Young Decl. ¶ 22. As a result, Factor VIII replacement therapy is usually not effective in the inhibitor population. *Id.* ¶ 23; Krishnaswamy Rpt. ¶ 29.

Before the introduction of Hemlibra, the inhibitor population had few effective treatment options. *See* Young Decl. ¶¶ 22–27; Malackowski Rpt. at 21. One option was a therapy called "Immune Tolerance Induction (ITI)." Young Decl. ¶ 24; Malackowski Rpt. at 21. But that treatment is costly, complicated, and prolonged, requiring daily intravenous infusions of high concentrations of Factor VIII over the course of months or even years until the body's immune system begins to tolerate it, if ever. *See* Young Decl. ¶ 24; Malackowski Rpt. at 21.

As of 2018, inhibitor patients could also take one of two "bypass agents" (BPAs), including Baxalta's product FEIBA ("Factor Eight Inhibitor Bypassing Activity"). *See* Young Decl. ¶ 25; Malackowski Rpt. at 21. BPAs work by bypassing the Factor VIII step in the clotting cascade. *See* Young Decl. ¶ 25; Malackowski Rpt. at 21. Like Factor VIII replacement therapy, BPAs can be used in two ways: on-demand when a bleeding episode occurs and/or on a regular schedule as prophylaxis. Young Decl. ¶ 26; Malackowski Rpt. at 25. But they too must be infused, which

may impose a substantial treatment burden on patients and their families. In particular, the infusion can take up to an hour as often as every other day in order to achieve the desired prophylactic effect. *See* Young Decl. ¶ 27; Malackowski Rpt. at 34.

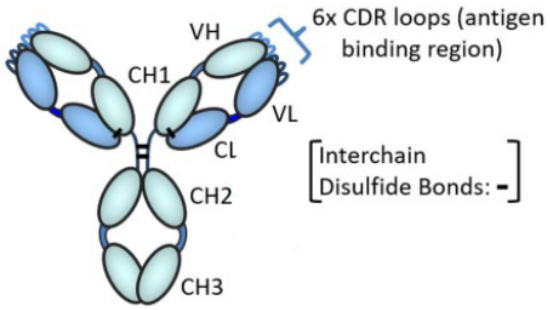
B. Baxalta's Search for an Antibody-Based Hemophilia A Treatment

Recognizing the drawbacks with all the existing treatment options, in 1998, scientists working for Baxalta's predecessor began experimenting with ideas for new, better hemophilia A treatments, particularly for inhibitor patients. Dr. Friedrich Scheiflinger, one of the '590 patent's named inventors, had the idea of using an antibody against Factor IX/IXa to increase the procoagulant activity of Factor IXa even in the absence of Factor VIII. *See* '590 patent, col. 2, ll. 29–44; Cole Decl. vol. 1, Ex. 11, Scheiflinger Dep. Tr. at 98:8–99:20, 101:02–12.

1. Antibody Structure and Genetic Modification

Antibodies are a key component of the immune system. Strohl Decl., ECF No. 413, Ex. 1, Opening Rpt., ¶ 35 (Strohl Rpt.). When confronted with a foreign molecule, or "antigen," the immune system's "B cells" (a type of white blood cell) generate antibodies that attack the antigen by binding to them. *See id.* ¶¶ 35, 41; Garcia Decl., ECF No. 415, Ex. 1, ECF No. 415-1, Opening Rpt. (Garcia Rpt.), ¶ 58. Each unique B-cell produces multiple copies of one specific antibody—meaning, the secreted antibody can bind to only one antigen. Garcia Rpt. ¶ 59. The binding of an antigen to the B-cell surface stimulates the B-cell to divide and mature into identical cells, secreting millions of antibodies into the bloodstream and lymphatic system. *See id.* ¶ 57. An antibody, as that term has been construed in the '590 patent, is "an immunoglobulin molecule having a specific amino acid sequence comprising two heavy chains (H chains) and two light chains (L chains)." *Baxalta*, 972 F.3d at 1349.

Antibodies can be visualized as forming a "Y" shape, with two arms connected by disulfide bonds. Strohl Rpt. ¶ 35. Each arm of the Y shape contains two polypeptides known as the heavy (H) chain and the light (L) chain. *See id.* Each of its heavy and light chains consist of two regions. *See id.* ¶ 36. The portions of the heavy and light chains that vary from antibody to antibody depending on the antigen are called the "variable domains," designated VH and VL, respectively. *Id.* ¶¶ 36–37. Variable regions include (i) complementarity-determining regions ("CDRs"), which are amino acid sequences that play a key role in the antibody's binding to an antigen, and (ii) framework regions, which serve as "scaffolds for the CDRs." *Id.* ¶¶ 36, 39. The remaining portions are called Constant (C) regions of each chain. *See id.* ¶ 36. This is a schematic of an antibody:



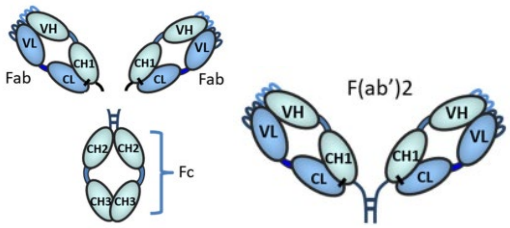
Id. ¶ 35 (Figure 1).

Based on the structure of the constant regions, antibodies are grouped into five classes—IgA, IgD, IgE, IgG, and IgM—each with closely related but different functions. *Id.* ¶ 37.[3] The constant region of all antibodies of the same isotype are identical (*e.g.*, all IgG antibodies have the same constant region and that constant region differs from that of IgA antibodies). *Id.* Because

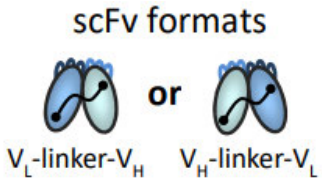
[3] "Ig" stands for immunoglobulin, and each letter signifies the specific class, which may change depending on the stage of the immune response. *See* Marasco Rpt. ¶ 71. The various antibody isotypes "differ from one another in biological properties, functional locations, and ability to deal with different antigens." Marasco Rpt. ¶ 278. IgGs are the most prevalent class, whereas IgDs are the least prevalent. *See* Strohl Rpt. ¶ 37.

each arm of a naturally occurring antibody is identical, each arm targets the same antigen. *Id.* ¶ 38. Naturally occurring antibodies are thus said to be "monospecific." *Id.*

Scientists have developed various genetic engineering techniques for altering natural antibodies to make a wide variety of molecules. Some are of different sizes than natural antibodies, whereas others have different binding specificities or different constant-region functions. For example, scientists have used protein-cleaving enzymes to cut antibodies into "antibody fragments." *Id.* ¶¶ 46–47. These include the Fab, Fc, and F(ab')$_2$ fragments, shown below.

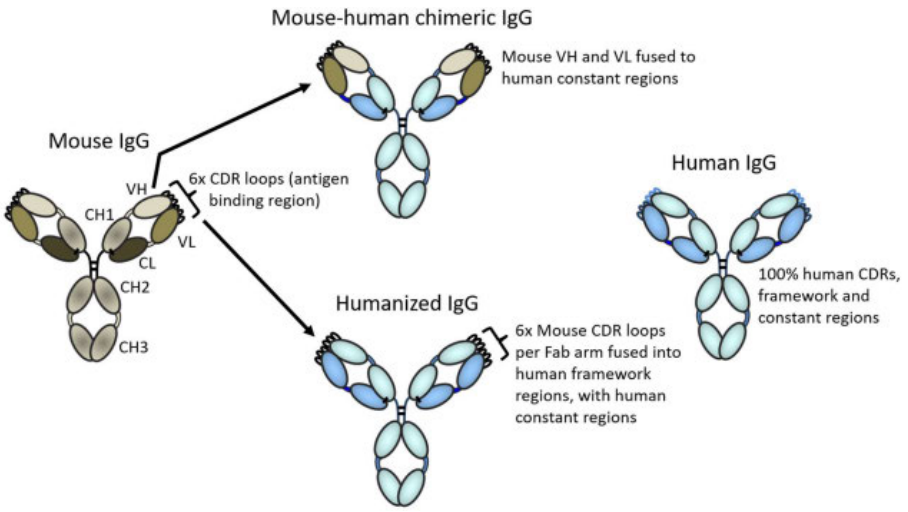


Id. at Figs. 2–3. They have also used recombinant DNA techniques to derive antibody fragments beyond simple enzymatic cleavage of a full-length antibody. One example (depicted below) is a fragment called a single-chain Fv (scFv), which contains the variable region of a heavy chain and the variable region of a light chain, held together by a synthetic string of amino acids. *Id.* ¶ 48.



Id. at Fig. 4.

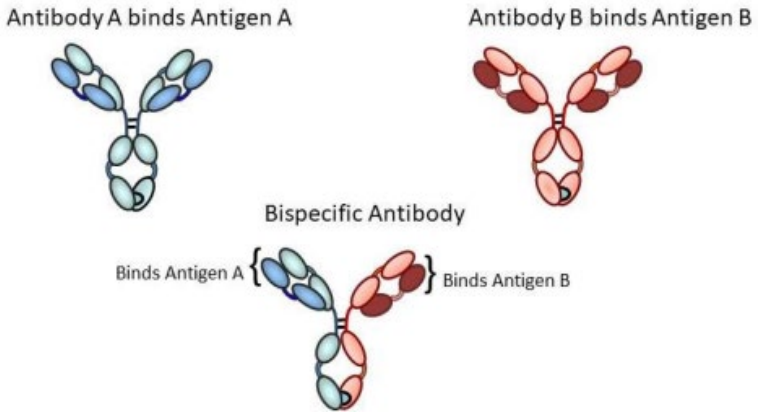
Genetic engineering has also made possible the construction of antibodies that are part animal sequence and part human sequence. *Id.* ¶ 49. These are known as "chimeric" and "humanized" antibodies—as shown below. *Id.*



Id. at Fig. 5. The benefit of humanizing antibodies is that it lessens the chances of an immune response to the antibody. *Id.* ¶ 50. In early efforts to use mouse (or murine) antibodies as therapeutic agents in humans, scientists observed that the human immune system recognized the murine antibody as a foreign substance and made antibodies against it. *Id.* This became known as the "HAMA response," for "human anti-mouse antibody." *Id.* It spurred research to develop a way to engineer antibodies containing more human amino acid sequences and less animal (*e.g.*, murine) sequences. *Id.* ¶ 51.

Initially, scientists used genetic engineering techniques to create "chimeric antibodies" by splicing together genetic material (DNA) encoding the variable regions of animal antibodies (usually murine) with DNA encoding the constant regions of human antibodies. *Id.* ¶¶ 52–53; '590 patent, col. 6, l. 64–col. 7, l. 3. Although successful at first, over time it became clear that humans were developing a HAMA response to the murine sequences in the chimeric antibodies. Strohl Rpt. ¶ 54. To avoid that response, scientists designed "humanized antibodies" wherein non-human CDRs are inserted into an otherwise-human antibody. '590 patent, col. 6, ll. 49–57. In the resulting antibody, the binding affinity is preserved, while adverse human immune reaction is significantly reduced as compared to the original animal antibody. Strohl Rpt. ¶¶ 55–56.

Finally, scientists have created "bispecific antibodies" by pairing the heavy and light chains of an antibody that binds to one antigen with the heavy and light chains of a different antibody that binds to a different antigen. *Id.* ¶ 61. The resulting antibody, depicted below, is thus capable of binding two antigens. *Id.*; '590 patent, col. 7, ll. 32–34.



Strohl Rpt. at Fig. 7.

2. The '590 Patent

Against this backdrop, in 1998, the scientists at Baxalta were experimenting with the idea of using an antibody binding to Factor IX/IXa to increase the procoagulant activity of Factor IXa even in the absence of Factor VIII. '590 patent, col. 2, ll. 29–44. Over the course of approximately four years, they used hybridoma techniques (described below) to create monospecific antibodies that bind to Factor IX or IXa and increase the procoagulant activity of Factor IXa. Cole Decl. vol. 1, ECF No. 409 ("Cole Decl. vol. 1"), Ex. 13, Kerschbaumer Dep. Tr. at 14:325–15:328; '590 patent, col. 7, l. 65–col. 8, l. 1; col. 9, l. 66–col. 10, l. 37; Sheehan Rpt. ¶ 166.

The '590 patent, titled "Factor IX/factor IXa activating antibodies and antibody derivatives," was filed on September 14, 2000, issued on April 25, 2006, and expired in December 2021. *See* Def.'s Mot. at 10. In total, the '590 patent discloses eleven working examples of

antibodies that bind to Factor IX/IXa and increase the procoagulant activity of Factor IXa.[4] Pl.'s Opp'n, Ex. 4, Marasco Rebuttal Rpt., ECF No. 424-5, ¶ 124 (Marasco Rpt.), ¶ 111. To find the eleven examples, the inventors tested tens of thousands of antibodies in assays designed to measure Factor VIII-like activity. *See* '590 patent, col. 10, l. 39–col. 12, l. 56; Joint Stip. ¶ 10. The examples are all murine, monospecific antibodies of the IgG and IgM isotypes, as well as a number of scFv fragments from some of those antibodies, and one Fab fragment. *See* Marasco Dep. Tr. at 102:12–126:04.

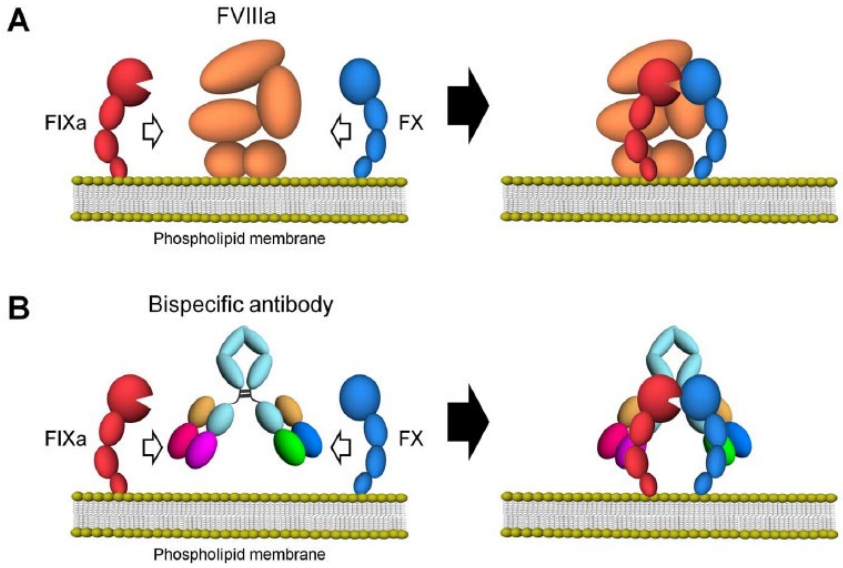
Baxalta's efforts to produce antibodies that bind to Factor IX/IXa and increase the procoagulant activity of Factor IXa continued until 2003. *See* Cole Decl. vol. 1, Ex. 6, Baxalta's Suppl. Resp. to Interrog. 11, at 47. The parties agree that Baxalta has never commercialized an antibody for the treatment of hemophilia A in inhibitor patients consistent with the stated purpose of the '590 patent. *See* Scheiflinger Dep. Tr. at 48:25–49:08; Sheehan Rpt. ¶ 166; Garcia Rpt. ¶ 221; *see also* '590 patent, col. 2, ll. 25–28 ("[I]t is an object of the [] invention to provide a preparation for the treatment of blood coagulation disorders which has particular advantages for factor VIII inhibitor patients[,] . . . through the use of antibodies . . . against factor IX/IXa.").

C. Chugai's Invention of Emicizumab

Around the same time that Baxalta's scientists were experimenting with antibodies capable of binding to Factor IX and increasing the procoagulant activity of Factor IXa, scientists in Japan at Chugai were also working to develop antibody-based treatments for hemophilia A. By at least October 27, 2000, Chugai had the idea of using a humanized bispecific antibody that would bind Factor IXa with one arm and Factor X with the other, holding Factor IXa and Factor X in a position

[4] The parties dispute whether the patent discloses 11 examples, but for the purposes of its motion for summary judgment, Genentech accepts that the specification discloses 11 working examples. *See* Joint Stip. of Fact, ECF No. 437 (Joint Stip.) ¶ 14.

by which Factor IXa would activate Factor X, similar to how Factor VIIIa functions (depicted below). *See* Strohl Rpt. ¶ 115; Cole Decl. vol. 1, Ex. 7 at GNE-01138116 (dated Oct. 27, 2000); *id.*, Ex. 8, Kitazawa Dep. Tr. at 185:01–186:10, 215:20–25; *id.*, Ex. 9 at GNE-01137931; *id.*, Ex. 10 at GNE-01137957.



Sampei et al., *Identification and Multidimensional Optimization of an Asymmetric Bispecific IgG Antibody Mimicking the Function of Factor VIII Cofactor Activity*, PLoS ONE 8(2):1–13 (2013) (Sampei Article), at 2, Fig. 1 (showing (A) Factor VIIIa forming a complex with Factor IXa and supporting the interaction between Factor IXa and Factor X through its binding ability to both factors on the phospholipid membrane, and (B) A bispecific antibody (like emicizumab) binding to Factor IXa and Factor X, promoting the interaction between Factor IXa and Factor X and therefore exerting Factor VIII mimetic activity on the phospholipid membrane.).

Genentech's expert, Dr. William R. Strohl, details the lengthy trial-and-error process through which Chugai's scientists generated tens of thousands of combinations of Factor IX and Factor X antibodies, combining them into bispecific antibodies and testing them in assays. Strohl

Rpt. ¶¶ 202–224.[5] Once the scientists had discovered a candidate worthy of testing in animals and then in humans, they engaged in antibody engineering to refine and optimize the candidate antibody. *Id*. ¶¶ 208–210; Strohl Decl., Ex. 2, ECF No. 413-1, Responsive Report, ¶¶ 61–127 (Strohl Resp.). In total, "it took 10 or more full-time Chugai researchers almost 10 years to construct a therapeutically useful bispecific antibody that binds Factor IX/IXa with one arm and Factor X with the other." Strohl Rpt. ¶ 224.

The resulting antibody, emicizumab, is a humanized bispecific antibody that mimics the function of Factor VIIIa by binding to Factor IXa with one of its arms and to Factor X with the other. Young Decl. ¶ 28; Strohl Resp. ¶ 29. It is the active ingredient in Hemlibra—the first and only FDA-approved product for hemophilia A patients that can be injected under your skin (subcutaneously). Young Decl. ¶ 28; Malackowski Rpt. at 38. Hemlibra has been shown to increase procoagulant activity to about 10% of normal Factor VIII levels. *See* Decl. of Stephanie A. Smith, ECF No. 420, Ex. 1 at ¶ 49 (citing Uchida et al., *A first-in-human phase 1 study of ACE910, a novel factor VIII-mimetic bispecific antibody, in healthy subject*, Blood 127(13):1633–1641 (2016)); *Id.* at Ex. 2, ¶ 44. This is enough to move a patient from severe hemophilia A (with observed Factor VIII activity less than 1%) to at least a moderate category (with observed factor VIII activity between 1–5%) or even a mild category (with observed factor VIII activity between 5–40%). *See* Smith Decl., Ex. 2, ¶ 44; Krishnaswamy Rpt. ¶ 117 (showing severity classifications).

[5] The work conducted by Chugai's scientists to discover emicizumab also is documented in literature. *See* Sampei Article; *see also* Sampei et al. (2013) Discussion, *available at https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0057479* (explaining that "the lead bispecific antibody was identified from approximately 40,000 different bispecific antibodies" and "[b]ispecific antibodies meeting the criteria for FVIII cofactor activity were extremely rare (<0.3%)").

On November 16, 2017, the FDA approved Hemlibra for routine prophylaxis to prevent or reduce the frequency of bleeding episodes in adult and pediatric patients with hemophilia A with Factor-VIII inhibitors. Young Decl. ¶ 28. On October 4, 2018, the FDA approved Hemlibra for non-inhibitor patients. *Id.*

V. Enablement Standard

A patent claim is presumed enabled unless proven otherwise by clear and convincing evidence. 35 U.S.C. § 282; *Ormco Corp. v. Align Tech., Inc.*, 498 F.3d 1307, 1318 (Fed. Cir. 2007). The central question for enablement is whether the specification enables the full scope of its claims without undue experimentation. *Plant Genetic Sys., N.V. v. DeKalb Genetics Corp.*, 315 F.3d 1335, 1339 (Fed. Cir. 2003). "Enablement is not precluded where a 'reasonable' amount of routine experimentation is required to practice a claimed invention." *ALZA Corp. v. Andrx Pharm., LLC*, 603 F.3d 935, 940 (Fed. Cir. 2010). To evaluate whether the patent enables a person of ordinary skill in the art to practice the invention without undue experimentation, courts consider a non-exclusive list of items, often referred to as the *Wands* factors: "(1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims." *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). A court need not consider each of the *Wands* factors, for they "are illustrative, not mandatory." *Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1213 (Fed. Cir. 1991).

VI. Undisputed Facts Relevant to Enablement

The following are undisputed material facts, based on the patent claims, the specification, the court's claim construction order, Genentech's Motion for Summary Judgment, Baxalta's response, and the parties' stipulation dated December 3, 2021:

1. Claim 1 of the '590 patent encompasses any isolated antibody or antibody fragment thereof that binds Factor IX or Factor IXa and increases the procoagulant activity of Factor IXa by any amount. *See* Claim Construction Order at 29.

2. The number of candidate antibodies or antibody fragments within the scope of claim 1 is high, "encompassing millions of different structural formats, binding epitopes, binding affinities, and mechanisms of action." Garcia Rpt. ¶ 215. The specification discloses working examples of only eleven antibodies that satisfy claim 1. *See* Joint Stip. ¶ 14.

3. The structural breadth of claim 1 is illustrated by its dependent claims. The dependent claims show that claim 1 is not specific to any particular isotype of the antibody or antibody fragment and includes antibodies that have been genetically engineered into different structural formats.

    a. Dependent claims 3 and 20, define *Markush* groups[6] and include antibodies of the IgA, IgE, IgG and IgM isotypes that are within the scope of claim 1. Claim 1 also includes the fifth principal isotype, IgD, because claim 1 is not limited by isotype. *See* Def.'s Mot. at 18; Marasco Dep. Tr. at 103:12–104:9. The patent does not disclose working examples of three of these isotypes: IgD, IgA,

[6] "A Markush group lists specified alternatives in a patent claim, typically in the form: a member selected from the group consisting of A, B, and C." *Gillette Co. v. Energizer Holdings, Inc.*, 405 F.3d 1367, 1372 (Fed. Cir. 2005) (citing to *Manual of Patent Examining Procedure* § 803.2 (2004)).

and IgE. *See* Marasco Dep. Tr. at 102:08–22; 105:06–09; 105:20–106:12. The two isotypes represented by working examples are those that are most commonly present in high proportion in the blood at the early stages of an immune response. Marasco Rpt. ¶ 71; Strohl Rpt. ¶ 37. Using the teachings of the '590 patent, it would be rare to discover antibodies of the IgA and IgE isotypes, as the vast majority of antibodies, 75 percent, exist as IgG and IgM isotypes. It would be rarer still to discover IgD antibodies using the teachings of the '590 patent, since they do not circulate in the bloodstream but are instead bound to the exterior membranes of immune-system cells. *See* Marasco Dep. Tr. at 104:13–105:5.

b. Dependent claims 4 and 19 define *Markush* groups that include various types of antibodies, namely monoclonal antibodies, chimeric antibodies, humanized antibodies, single chain antibodies (such as scFvs), bispecific antibodies, and diabodies, as well as dimers, oligomers, and multimers thereof. The '590 patent does not disclose working examples of seven of the nine structural formats in the *Markush* group of claims 4 and 19: chimeric antibodies, humanized antibodies, bispecific antibodies, diabodies, and dimers, oligomers and multimers thereof. *See* Marasco Dep. Tr. at 124:24–125:12,126:02–04; Scheiflinger Dep. Tr. at 66:16–17.

4. Claim 1 also is functionally broad. An antibody that increases the amount of procoagulant activity by the same amount as Factor VIII does (at least 40%) is within the scope of the asserted claims. *See* Marasco Dep. Tr. at 236:12–18; Cole Decl. vol. 2, ECF No. 410-1, Ex. 20, Krishnaswamy Dep. Tr. at 213:17–214:11. The highest estimated amount by which

any antibody disclosed in the '590 patent increased the procoagulant activity of Factor IXa was by 3.75% (antibody 198/A1)—far less than 40%.[7] *See* Krishnaswamy Rpt. ¶¶ 122–123. The 3.75% would only be capable of moving a patient with hemophilia A classified as severe to a moderate classification, pursuant to the below chart upon which both parties' experts rely.

Factor VIII Activity	**Classification**
< 0.01 IU/mL (< 1% of normal)	Severe
0.01 – 0.05 IU/mL (1%–5% of normal)	Moderate
> 0.05 – < 0.40 IU/mL (>5% – <40% of normal)	Mild

See Krishnaswamy Rpt. ¶¶ 117; Sheehan Rpt. ¶ 99. The specification does not disclose an antibody or antibody fragment that is therapeutically useful for moving someone suffering from a severe case of hemophilia A to a mild case. Patients with severe conditions represent about 60 percent of hemophilia A cases. Malackowski Rpt. at 22.

5. Claim 1 also includes antibodies or antibody fragments that are capable of increasing the procoagulant activity of Factor IXa in the presence of inhibitors (as specified in claim 2). The specification states that the objective of the patent is "to provide a preparation for the treatment of blood coagulation disorders which has particular advantages for factor VIII inhibitor patients." '590 patent, col. 2, ll. 25–28; *id.* at col. 2, ll. 29–45.

6. The '590 patent discloses only one working example of an antibody shown to increase the procoagulant activity of Factor IXa in the presence of Factor VIII inhibitors as required by claim 2 (antibody 193/AD3). *See* Marasco Dep. Tr. at 122:06–11; Marasco Rpt. ¶ 73. The highest amount that the 193/AD3 antibody increased the procoagulant activity of Factor

[7] The 3.75% figure does not appear in the specification; a person of ordinary skill in the art reading the specification could derive it from the information disclosed in Figure 25. *See* Krishnaswamy Rpt. ¶ 123.

IXa by was only 0.3–0.4% equivalent Factor VIII activity—a marginal amount. *See* Sheehan Rpt. ¶ 99; Krishnaswamy Rpt. ¶ 118.

7. In order to treat hemophilia A without a HAMA response, it would be necessary to utilize a humanized, or at least chimeric, antibody. *See* Strohl Rpt. ¶¶ 50–56.[8] There are no working examples of humanized or chimeric antibodies disclosed in the patent specification. *See* Marasco Dep. Tr. at 124:24–125:12,126:02–04; Scheiflinger Dep. Tr. at 66:16–17.

8. The inventors of the '590 patent performed their experimentation for a period of three to four years and never brought to market an antibody within the scope of claim 1 for the treatment of hemophilia A. *See* Scheiflinger Dep. Tr. at 48:25–49:08; Kerschbaumer Dep. Tr. at 14:325–15:328; Sheehan Rpt. ¶ 166.

9. Under the teachings of the '590 patent, arriving at an antibody that binds to Factor IX or IXa and increases the procoagulant activity of Factor IX is a multi-step process, involving experimentation at every critical step. *See infra* ¶¶ 10–27.

10. The level of skill in the art for the '590 patent is high,[9] Pl.'s Opp'n at 28–29 (citing Marasco Rpt. ¶¶ 264–265), and a person of ordinary skill in the art would be familiar with the

---

[8] *See also* Morrison, et al., *Chimeric human antibody molecules: mouse antigen- binding domains with human constant region domains*, Proc. Natl. Acad. Sci. USA 81, 6851-55 (1984), *cited in* Marasco Rpt. ¶ 286 n.254.

[9] There is no material difference between the parties' descriptions of the level of skill in the art. *See* Garcia Decl. ¶¶ 52, 53. The court adopts Baxalta's definition for the purposes of this motion. That is, a person of ordinary skill in this art is one who:

> would have had an advanced degree and relevant work experience, either an M.D. and several years' experience practicing in the area of hematology or a Ph.D. in a chemical science- or biological science-related discipline. This person would have a working knowledge of experimental methodologies for detecting the activity of factors in the clotting cascade, measuring blood clotting

technology and techniques discussed in the patent for producing and testing antibodies generally, Marasco Rpt. ¶¶ 264–265. But it would not be possible for a person skilled in the art to predict which antibodies would satisfy the claim limitations without trial-and-error testing. *See* Scheiflinger Dep. Tr. at 92:15–93:3; Marasco Dep. Tr. at 205:04–19; Krishnaswamy Dep. Tr. at 168:09–168:19.

11. There is no guidance or direction in the specification of the '590 patent as to how to identify antibodies that satisfy the claim limitations except by using trial and error. *See* Marasco Dep. Tr. at 205:04–19; Krishnaswamy Dep. Tr. at 168:09–168:19; Scheiflinger Dep. Tr. at 92:15–93:3.

12. "The only way to know [what antibodies bind as well as function as needed] is to make antibodies and test them." Krishnaswamy Dep. Tr. at 168:20–169:02; Marasco Dep. Tr. at 218:23–219:04.[10] The '590 patent does not describe what structural or other features of the disclosed antibodies cause them to bind to Factor IX/IXa or to increase the procoagulant activity of Factor IXa. *See* Garcia Rpt. ¶ 130; Scheiflinger Dep. Tr. at 91:23–92:3; 97:23–98:02.

> capabilities by a variety of means, or would have general familiarity with basic concepts in immunology, including basic knowledge of methods for making antibodies and using them as therapeutics. This hypothetical person would be teamed with or have access to other highly skilled individuals with advanced degrees (*e.g.*, Ph.Ds.) in other biological disciplines such as immunology or molecular biology who had several years' experience with methods to produce antibodies that bind to antigens of interest.

Id. ¶ 53; Marasco Rpt. ¶ 264.

[10] "Q. . . . [T]he only way that the patent teaches a person of ordinary skill how to tell whether a given antibody to Factor IX or Factor IXa, in fact, increases the procoagulant activity of Factor IXa is to test that antibody in an assay, correct? A. That's what the patent teaches."

13. At step one of the multi-step process for producing antibodies that bind to Factor IX or IXa and increase the procoagulant activity of Factor IXa, the specification discloses how to produce antibodies using one of several methods known in the prior art "(e.g., by conventional hybridoma techniques, or by means of phage display gene libraries, immunoglobulin chain shuffling or humanizing)." '590 patent, col. 7, l. 66–col. 9, l. 10.

14. The antibodies disclosed in the '590 patent were generated using the "hybridoma" technique, as shown in Example 1. *See* Joint Stip. ¶¶ 1, 6; '590 patent, col. 9, l. 66–col. 10, l. 37.

15. In the hybridoma process, mice in groups of one to three are injected with Factor IX or IXa over a period of days. Joint Stip. ¶¶ 3, 7; '590 patent, col. 9, l. 66–col. 10, l. 8.

16. The mouse's immune system responds to the Factor IX injections by producing B-cells in its spleen that secrete antibodies against the human antigen. Joint Stip. ¶ 3. Each B-cell produces only a single antibody. *Id.* It is not possible to predict whether a mouse used to make hybridomas will produce antibodies that satisfy the claim requirements. *See* Scheiflinger Dep. Tr. at 92:15–93:03.

17. Each mouse is then euthanized, and its spleen cells removed. *See* '590 patent, col. 10, l. 8–9; Joint Stip. ¶ 4. In order to enable murine antibodies to survive and to replicate themselves sufficiently for further experimentation, the spleen cells are fused with myeloma (cancer) cells through a process known in the prior art. '590 patent, col. 10, l. 9–11; Joint Stip. ¶ 5. The inventors performed and disclosed in the '590 patent at least four such "fusion" experiments, which they labeled #193, 195, 196, and 198. Joint Stip. ¶ 8 (citing '590 patent, col. 10, ll. 11–13).

18. In each fusion experiment, after the B-cells were fused with the myeloma cells, the resulting hybrid cells, or "hybridomas," were isolated and screened using techniques known in the prior art to determine whether they produce antibodies that bind to the antigen of interest (in this case, Factor IX or IXa). *See* Joint Stip. ¶¶ 5, 9; Garcia Rpt. at 30, Fig. 8; '590 patent, col. 10, ll. 14–31.

19. Not all of the antibodies produced at step one will bind to Factor IX/IXa. Oral Arg. Hr'g Tr., ECF No. 431 (Hr'g Tr.), at 28:16–18; Scheiflinger Dep. Tr. at 92:15–93:3. Around "60% of the hybridoma cell lines screened expressed an FIX-binding antibody." Garcia Decl., Ex. 2, Reply Rpt. ¶ 21 n. 15 (citing Scheiflinger, F. et al., *Enhancement of the enzymatic activity of activated coagulation factor IX by anti-factor IX antibodies*, J. Thromb Haemost 6(2):315–322 (2008)).

20. Once the antibodies are filtered to only those that bind to factor IX/IXa, they must undergo additional screening to determine which among them demonstrate the ability to increase the procoagulant activity of Factor IXa. Garcia Rpt. ¶ 213; Marasco Dep. Tr. at 218:23–219:04.

21. In terms of the method used to measure procoagulant activity, the patent provides that "all the methods used for determining Factor VIII activity may be used." '590 patent, col. 9, ll. 22–25; *see also* Claim Construction Order at 29 (construing "increases the procoagulant activity of Factor IXa" to mean "[t]he ability of Factor IXa to activate Factor X to Factor Xa by any amount as determined *by any assay used* to measure Factor VIII-like activity" (emphasis added)).

22. The specification of the '590 patent recommends the use of a modified version of the commercially available chromogenic test-kit called COATEST VIII:C/4® (COATEST)

for the hybridoma screening step. *See* '590 patent, col. 10, l. 40–col. 12, l. 56. The modified protocols disclosed in the examples of the '590 Patent are significantly different from the recommended protocol for the commercially available COATEST test. Sheehan Rpt. ¶ 150; Krishnaswamy Rpt. ¶¶ 141, 157–161. The modifications that the inventors made to the test were designed to make the test more sensitive. *See* Marasco Dep. Tr. at 219:5–15. They also made the test more complex and time consuming. *See* '590 patent, col. 10, ll. 48–67, col. 15, ll. 44–45; Krishnaswamy Rpt. ¶ 158; Sheehan Rpt. ¶ 150. Whereas the standard COATEST assay takes minutes, the modification disclosed in the patent takes several hours. *See* Sheehan Rpt. ¶ 150, Krishnaswamy Rpt. ¶ 158; '590 patent, col. 10, ll. 48–67, col. 15, ll. 44–45.

23. The inventors of the '590 patent did not use any of the other commonly used methods to screen hybridoma cells for procoagulant activity and did not determine or describe in the specification what modifications would be necessary for those other tests to function. '590 patent, col.9, ll. 22–25; Garcia Rpt. ¶ 220.

24. In an article published by the inventors after the '590 patent was filed, they disclosed that the vast majority of antibodies produced and screened in experiments leading up to the '590 patent (98.4% of them) did not increase the procoagulant activity of Factor IXa by any amount. *See* Cole Decl. vol. 2, Ex. 24, Scheiflinger, et al., *Enhancement of the Enzymatic Activity of Activated Coagulation Factor IX by Anti-Factor IX Antibodies* (Scheiflinger Article), at 320; Marasco Dep. Tr. at 202:18–21 (agreeing that the inventors reported only 1.6% of the antibodies had procoagulant activity); Marasco Rpt. ¶ 262 ("the number [of antibodies] that would activate Factor IXa such that there is an increase in procoagulant activity, is a very, very minor sub-fraction.").

25. Once the inventors discovered antibodies that increased the procoagulant activity of Factor IXa by screening the hybridoma cells using the modified COATEST assay, they then tested one of those antibodies (193/AD3) in aPTT assays to measure clotting time, including in the presence of Factor VIII inhibitors. '590 patent, col. 16, l. 44–col. 17, l. 67; Figures 9, 10A, 10B.

26. There are no examples in the patent of seven of the nine structural formats falling under claim 4 (a chimeric antibody, a humanized antibody, a bispecific antibody, a diabody, or di-, oligo- or multimers thereof). *See* Marasco Dep. Tr. at 102:12–126:04. In order to arrive at those antibody formats from the antibodies produced through the aforementioned steps, it would be necessary for one skilled in the art to genetically modify them. *See supra* § IV.B.1. This was never done, and the patent does not provide specific guidance on how such modification would take place. Although a person skilled in the art would be familiar with the procedures for modifying antibodies using techniques known in the prior art, additional confirmatory testing would have been necessary following modification to ensure that the binding and activating functions of the antibody remained in place. *See* Marasco Rpt. ¶¶ 235, 275; *see also* Garcia Rpt. ¶ 212; Marasco Dep. Tr. at 127:24–128:25.

27. For example, the process for humanizing antibodies was well-known in the art prior to 1999, *see* Marasco Rpt. ¶ 289 n. 258 (citing Strohl Dep. Tr., ECF No. 424-11, at 17:24–18:9), but the process was "not as efficient [] as sometimes presented," Marasco Dep. Tr. at 130:18–19. The process involves selecting "human framework regions . . . from heavy chain and light chain sequences of over 1,000 human sequences each," *id*. at 130:20–22, and "the resulting antibody, despite having the same variable region as the murine antibody, frequently does not have the same effectiveness as the original murine antibody,"

id. at 130:23–131:01. Given this uncertainty, additional screening would be required to confirm whether there had been any degradation in the binding or activating functions of the antibody. *See id.* at 132:10–12; Marasco Rpt. ¶ 275.

28. The accused product, emicizumab, is a bispecific humanized antibody that mimics Factor VIIIa by binding Factor IXa with one arm and Factor X with the other arm. *See* Sampei Article at 1. It took the scientists at Chugai almost 10 years to develop emicizumab. Strohl Rpt. ¶ 224. They underwent a multi-phased, trial-and-error process that involved screening tens of thousands of antibodies and engineering the resulting antibodies for optimization before finding one that was suitable for clinical use. *Id.* ¶¶ 212–224; *see also* Sampei Article at 1–13. "The lead bispecific antibody was identified from approximately 40,000 different bispecific antibodies" and "[b]ispecific antibodies meeting the criteria for FVIII[-like] activity were extremely rare (<0.3%)." Sampei et al. (2013) Discussion, *available at https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0057479.*

VII. Application of the *Wands* Factors

Applying the *Wands* factors here, the court finds as a matter of law that undue experimentation would be needed to practice the full scope of the claimed invention. First, with respect to "the quantity of experimentation necessary" (factor 1), Baxalta does not dispute that practicing the teachings of the '590 patent involves a large amount of experimentation. The potential candidates number in the millions. *See supra* § VI ¶ 2. As discussed, the patent teaches a multi-step process, with screening at every critical step to determine antibodies within the scope of the claims. *See id.* ¶¶ 13–27. Turning to factor 2, there is a limited "amount of guidance presented in the patent." *See id.* ¶ 11. There is no guidance or direction as to how to identify antibodies that satisfy the claims' limitations other than by utilizing trial and error. *See id.* This

lack of guidance is compounded by a limited number of "working examples" (factor 3). While the specification of the '590 patent discloses eleven working examples, they are all monospecific murine antibodies or fragments thereof that bind to Factor IX or IXa and increase the procoagulant activity of Factor IXa by a small amount. *See id.* ¶¶ 1–4. It does not disclose working examples of antibodies of the IgE, IgA, or IGD isotypes; of humanized, chimeric, or bispecific antibodies; of diabodies; or of dimers, oligomers or multimers thereof. *See id.* ¶ 3. There also is no working example of an antibody that increases the procoagulant activity of Factor IXa by an amount capable of moving a patient with a severe case of hemophilia A to a mild case. *See id.* ¶ 4. There is not a single example of an antibody that produces procoagulant activity in the presence of Factor VIII inhibitors by more than a marginal amount. *See id.* ¶¶ 4–6.

Courts often consider factors 4 and 7 (the "nature of the invention" and the "predictability or unpredictability" of the art) together. *See, e.g.*, *Alza Corp. v. Andrx Pharms., LLC*, 607 F. Supp. 2d 614, 655–56 (D. Del. 2009). This area of art is inherently unpredictable. The field of antibodies is itself unpredictable. *See Centocor Ortho Biotech, Inc. v. Abbott Labs.*, 636 F.3d 1341, 1352 (Fed. Cir. 2011) (analogizing finding an appropriate antibody for a particular antigen to searching for a key "on a ring with *a million* keys on it" (internal citations and quotation marks omitted)). That unpredictability is compounded here by the lack of guidance as to how to produce antibodies satisfying the full scope of the claims other than by trial-and-error. *See Fisher*, 427 F.2d at 839 ("In cases involving unpredictable factors, such as most chemical reactions and physiological activity, the scope of enablement obviously varies inversely with the degree of unpredictability of the factors involved.").

Turning to factor 6—the "relative skill of those in the art"—the level of skill would be high, with a POSITA holding "an advanced degree and relevant work experience, either an M.D.

and several years' experience practicing in the area of hematology or a Ph.D. in a chemical science- or biological science-related discipline." *Supra* note 10. Given the "state of the prior art" (factor 5), a POSITA would be familiar with the techniques for producing antibodies using hybridoma or phage display technology and in using the standard chromogenic or aPTT assays used in the trial and error process, *see supra* § VI ¶ 10, but could not predict in advance which antibodies would satisfy the claim limitations, *see id.* ¶ 11.

Finally, turning to factor 8, the undisputed facts show that a reasonable factfinder could only find that the "breadth of the claims" is great. By Baxalta's experts' own admissions, claim 1 covers all antibodies and fragments of any format, isotype or subtype, that bind with any affinity to factor IX/IXa, that achieve procoagulant effect through any mechanism of action, and that demonstrate procoagulant activity ranging from minuscule to therapeutically useful amounts, with or without the presence of Factor VIII inhibitors. *See id.* ¶¶ 1–5.

Decisions of the Federal Circuit applying the *Wands* factors make clear that the claims asserted here are not enabled.

VIII. Enablement of the Full Scope of the Asserted Claims of the '590 Patent

A. Make-and-Screen Nature of Invention

First, where, as here, there are a large number of potential candidates, few working examples disclosed in the patent, and no guidance in the specification as to how to practice the full scope of the invention except to use trial and error to narrow down the potential candidates to those satisfying the claims' functional limitations—the asserted claims are not enabled. *See Idenix Pharms. LLC v. Gilead Scis. Inc.*, 941 F.3d 1149, 1155–56 (Fed. Cir. 2019) (finding nonenablement where the claims included the broad functional limitation of having efficacy against hepatitis C virus, which required screening a large number of candidates to identify

compounds that satisfied the limitation); *Enzo Life Scis., Inc. v. Roche Molecular Sys., Inc.*, 928 F.3d 1340, 1346–47 (Fed. Cir. 2019) (finding nonenablement where the claims required both a particular structure and functionality but the specification failed to teach one of skill in the art whether the many embodiments of the broad claims would exhibit that required functionality); *Wyeth & Cordis Corp. v. Abbott Labs.*, 720 F.3d 1380, 1385–86 (Fed. Cir. 2013) (finding, due to the large number of possible candidates within the scope of the claims and the specification's corresponding lack of structural guidance, it would have required undue experimentation to synthesize and screen each candidate to determine which compounds in the claimed class exhibited the claimed functionality); *see also McRO*, 959 F.3d at 1100 n.2 ("In cases involving claims that state certain structural requirements and also require performance of some function (*e.g.*, efficacy for a certain purpose), we have explained that undue experimentation can include undue experimentation in identifying, from among the many concretely identified compounds that meet the structural requirements, the compounds that satisfy the functional requirement.").

In this respect, the facts of this case are strikingly similar to the facts of *Amgen Inc. v. Sanofi, Aventisub LLC*, 987 F.3d 1080 (Fed. Cir. 2021). There, as here, the claims were directed to a genus that was claimed broadly in terms of functionality. The two patents at issue there were directed to monoclonal antibodies for use in treatment of elevated low-density lipoprotein ("LDL") cholesterol—a leading cause of heart disease. *Amgen*, 987 F.3d at 1082–83. The body removes LDL cholesterol from the blood stream using LDL receptors. *Id.* at 1082. But "PCSK9," a naturally occurring protein, can bind to LDL receptors and cause the receptors to be destroyed, an undesirable result. *Id.* at 1082–83. The antibodies disclosed in Amgen's patents were claimed to prevent the degradation of LDL receptors by binding a specific region of PCSK9. *Id.* at 1083. By binding that specific region, the antibodies block PCSK9 from binding LDL receptors and causing

them to be destroyed. *Id.* The asserted claims thus imposed a functional limitation that the antibodies bind to a specific target. [11] The specification disclosed working examples of 26 antibodies that satisfied the claim limitations. *Id.*

In assessing enablement, the court first explained there are "high hurdles in fulfilling the enablement requirement for claims with broad functional language." *Id.* at 1087. Applying that standard, the court agreed with the district court's finding that the specification did not enable preparation of the full scope of the asserted claims—that the antibodies bind a specific target. *Amgen*, 987 F.3d at 1087. Key to that determination was the court's finding that the "claims [we]re far broader in functional diversity than the disclosed examples," and "the only ways for a person of ordinary skill to discover undisclosed claimed embodiments would be through either 'trial and error, by making changes to the disclosed antibodies and then screening those antibodies for the desired binding and blocking properties,' or else 'by discovering the antibodies *de novo*' according to a randomization-and-screening 'roadmap.'" *Id.* at 1088 (quoting *Amgen Inc. v. Sanofi*, 2019 WL 4058927, at *11 (D. Del. 2019)); *see also Idenix*, 941 F.3d at 1161 ("A specification that requires a [POSITA] to 'engage in an iterative, trial-and-error process to practice the claimed invention' does not provide an enabling disclosure." (quoting *ALZA*, 603 F.3d at 941).

Amgen's reasoning applies with equal force here, where the asserted claims also set forth not one but two functional requirements: that the antibodies bind to a target (Factor IX or IXa) and alter that target's activity (increasing the procoagulant activity of Factor IXa). Marasco Rpt. ¶ 63 (agreeing with Genentech's expert, Dr. K. Christopher Garcia, that the claims include the two functional limitations). Even if the first functional requirement (binding) were enabled, the second

[11] Although the asserted claims also included a functional limitation of "blocking the PCSK9/LDLR interaction," *Amgen*, 987 F.3d at 1083, the court found that "[t]he binding limitation [alone] [wa]s [] enough [] to require undue experimentation," *id.* at 1087.

is not. The record shows that if a person of ordinary skill in the art (POSITA) started with the genus of antibodies and antibody fragments that bind to Factor IX or IXa, only a very tiny percentage of those will meet the claims' functional limitation that the antibody or antibody fragment increase the procoagulant activity of Factor IXa,[12] and the only way to find that small number within the larger whole (of potentially millions of combinations satisfying the structural requirements), given the inherent unpredictability of the art and the lack of guidance in the specification, is by screening tens of thousands (if not more) antibodies or antibody fragments for procoagulant activity. *See supra* § VI ¶¶ 2, 10–24. It is a search for a needle in a haystack.

While Baxalta does not dispute the breadth of the claims, it asserts that only a "very, very minor sub-fraction" of antibodies will satisfy the claim limitations. Pl.'s Opp'n at 12 (quoting Garcia Rpt. ¶ 189). That is true, but this only exemplifies how substantial experimentation is necessary to sift through the broad genus of possible candidates to find the narrow species that satisfy the claim limitation.

The Federal Circuit considered an almost identical theory in *Idenix*, where the court found the claims were invalid for lack of enablement. 941 F.3d at 1161–63. The patent in *Idenix* claimed a method of treatment for the hepatitis C virus (HCV) by using a particular pharmaceutical drug.

---

[12] Although the results of the '590 patent inventors' experimentation (showing only 1.6% of antibodies screened bound to Factor IX/IXa and increased the procoagulant activity of Factor IXa) are not disclosed in the patent, the court may consider extrinsic evidence of those results to support its finding of non-enablement. *See Pharm. Res., Inc. v. Roxane Labs., Inc.*, 253 F. App'x 26, 31 n.3 (Fed. Cir. 2007) ("Although extrinsic evidence cannot be used to supplement a non-enabling specification, such evidence can shed light on whether the specification is itself enabling."); *see also Atlas Powder Co. v. E.I. du Pont De Nemours & Co.*, 750 F.2d 1569, 1577 (Fed. Cir. 1984) (considering results of experiments performed by patentee prior to filing the patent); *Enzo Biochem, Inc. v. Calgene, Inc.*, 188 F.3d 1362, 1373 (Fed. Cir. 1999) (determining evidence of the patentee's own experimental failures was appropriate to consider).

The patent claimed the drug in a generic manner that included a large number of related compounds. The only independent claim of the patent recited:

> 1. A method for the treatment of a hepatitis C virus infection, comprising administering an effective amount of a purine or pyrimidine β-D-2'-methyl-ribofuranosyl nucleoside or a phosphate thereof, or a pharmaceutically acceptable salt or ester thereof.

Id. at 1155 (quoting U.S. Patent No. 7,608,597).

Of the "billions of potential 2'-methyl-up nucleosides," the specification identified only a small subset (four) compounds as being effective. *Id.* at 1156, 1161. The court thus found that the broad functional limitation of having efficacy against hepatis C virus (HCV) meant that there were a very large number of potential nucleoside candidates and only a few examples that satisfied the claim limitation. *Id.* at 1155–56, 1162. Idenix argued that the claims were not broad because, '[w]hen required to take all of the claim limitations into account, Gilead's witnesses described the claims as embracing only a 'small' number of compounds." *Id.* at 1162.

The court rejected that analysis as "backwards," explaining that "to get from a large number of candidate compounds to a relatively speaking small number of effective compounds . . . leaves a [POSITA] searching for a needle in a haystack to determine which of the 'large number' of . . . [candidate compounds] falls into the 'small' group of candidates that effectively treats HCV." *Id.* "The size disparity between those two groups," the court reasoned, "requires significant experimentation, which weighs against enablement, not for it." *Id.* The same reasoning applies here where the only way to find the very small number of antibodies that meet the claims' broad functional requirements, among millions of possible combinations, is to experiment. That there are few needles in the haystack makes the search harder, not easier.

Baxalta argues that that the experimentation required to practice the full scope of the invention is not undue here because "a POSITA need only engage in *routine* experimentation," as

shown in Examples 1 and 2 of the specification. Pl.'s Opp'n at 22 (emphasis added) (quoting Marasco Rpt. ¶ 214).[13] The same was true in *Amgen*, where expert testimony showed that "a person of skill in the art c[ould] make all antibodies within the scope of the claims by following a roadmap using anchor antibodies and well-known screening techniques as described in the specification or by making conservative amino acid substitutions in the twenty-six examples." *Amgen*, 987 F.3d at 1085. The court nevertheless found the experimentation was undue. *Id.* at 1088.

The Federal Circuit also considered and rejected a similar argument in *Wyeth*. Like Baxalta, Wyeth argued that practicing the full scope of the claims would not require undue experimentation because it "would have required only *routine* experimentation." 720 F.3d at 1384 (emphasis added). The Federal Circuit disagreed, explaining that even taking all of Wyeth's contentions—including that "one of ordinary skill could routinely use the assays disclosed in the specification to determine" which compounds fall within the scope of the claims, the claims were not enabled because "there [we]re still at least tens of thousands of candidates" to screen. *Id.* at 1385.

The same reasoning dooms Baxalta's routine experimentation arguments here. There is no guidance or direction in the specification of the '590 patent as to how to distinguish antibodies that bind to Factor IXa and increase the procoagulant activity of Factor IXa from those that do not. *Supra* ¶ 10. And "there is no genuine dispute that it would be necessary to first synthesize and then screen *each* candidate compound using the assays disclosed in the specification to determine

[13] Baxalta also points to its expert's statement that "'[t]he specification instructs a POSITA to use well-known, inexpensive, and efficient means of producing an antibody or fragment thereof that binds to Factor IX or Factor IXa and identifying those that increase the procoagulant activity of Factor IXa,' [and] it would not require 'substantial time and effort' to make and use the claimed invention." Pl.'s Opp'n at 22 (quoting Marasco Rpt. ¶ 241).

whether it has" procoagulant effect, and until you screen the antibodies, "you can't tell whether they work or not." *Wyeth*, 720 F.3d at 1385.

Finally, while acknowledging that "a POSITA would need to screen for procoagulant activity," Pl.'s Opp'n at 22 (citing Marasco Dep. Tr. at 204:01–205:19), Baxalta argues that the requisite experimentation is nevertheless not undue because "POSITAs would 'feel confident that they could [utilize the teaching of the patent to produce] antibodies [] that have procoagulant return,'" *id.* (quoting Marasco Dep. Tr. at 209:02–09). Baxalta quotes one of its experts' testimony that there is a "'profound[]' difference between (i) making antibodies that bind to Factor IX/IXa and screening them for procoagulant activity, and (ii) starting from scratch or through trial and error." *Id.* at 22 (quoting Marasco Dep. Tr. at 206:07–13); *see also* Marasco Dep. Tr. at 206:14–19 ("A. . . . It's no longer an unknown of maybe I'll find them."). But this was also true in the *Idenix*, *Enzo*, *Wyeth* and *Amgen* cases, where the inventors had identified a small number of compounds within the scope of the claims and the court found the claims were not enabled given the breadth of the claims and the lack of sufficient guidance in the specifications.[14] There is nothing in the specification teaching how to identify any antibodies complying with the claim limitations other than by repeating the same process the inventors used to identify the eleven examples disclosed in the specification.

[14] Baxalta also attempts to distinguish *Amgen* on the basis that the claims there specified the amino acids (or residues) on PCSK9 to which the antibodies must bind. Pl.'s Opp'n at 32 (citing *Amgen*, 987 F.3d at 1087 n.1 ("For example, there are three claimed residues to which not one disclosed example binds."")). Baxalta maintains "[t]here is no similar unpredictability or lack of enablement in the" asserted claims in this case because they "merely require binding to Factor IX/IXa and do not require binding to specific residues." Pl.'s Opp'n at 32. The court disagrees. Here, similar to *Amgen*, there are various categories of antibodies that are identified in the claims that are not represented by working examples.

B. Broad Functional Scope

Second, the patent claims in this case, even more so than those in *Amgen* (which focused only on the binding requirement), cover a wide range of functionality in terms of procoagulant activity and that range is not represented by working examples**.** The Federal Circuit's cases make clear that where, as here, "a range is claimed, there must be reasonable enablement of the scope of the range." *See Amgen*, 987 F.3d at 1085 (quoting *McRO, Inc. v. Bandai Namco Games Am. Inc.*, 959 F.3d 1091, 1100 (Fed. Cir. 2020)); *see also MagSil Corp. v. Hitachi Global Storage Techs., Inc.*, 687 F.3d 1377, 1384 (Fed. Cir. 2012) (claims not enabled where the patentee argued for a broad scope despite meager results achieved by the inventors). By Baxalta's own admission, the patent covers everything from a barely perceptible amount of procoagulant activity at the bottom end to an amount that would be created by Factor VIII itself (at least 40%) at the upper end. *See* Marasco Dep. Tr. at 236:12–18;[15] Krishnaswamy Dep. Tr. at 213:17–214:11;[16] *see also supra* § VI ¶ 4.

The Federal Circuit's decision in *MagSil* is instructive. There, a patentee asserted infringement of a claim directed to a device used in computer hard drive disks that required a "change in resistance by at least 10%" between two electrodes on the device. 687 F.3d at 1379–80. The background section of the patent explained that past efforts to "produce an adequate level of change in the [ ] resistance" had achieved only a 2.7% change. *Id.* at 1379. The Federal Circuit found the claims were not enabled. In relevant part, the court observed that the patent specification "only disclose[d] enough information to achieve an 11.8% resistive change," even

[15] "Q. So if an antibody binds Factor IX or IXa and increases the procoagulant activity of Factor IXa by as much as Factor VIII does, that antibody is within the scope of the claim, correct? A. . . . yeah, I think it's within the scope of the claim."

[16] "Q. . . . [I]f it turned out that . . . there were an antibody that mirrored the activity of factor VIII itself, such an antibody would still be covered by these claims if it met the other limitations; correct? A. I would agree with that statement, yes."

though the claims were construed to cover resistive changes "from 10% up to infinity." *Id.* at 1383. The Federal Circuit further stated, "[t]he record contains no showing that the knowledge of [a skilled] artisan would permit, at the time of filing, achievement of the modern values above 600% without undue experimentation." *Id.* at 1384. "Indeed," the court observed," it had taken "nearly twelve years of experimentation to actually reach those [modern] values." *Id.* The same problem exists here.

Just as it took twelve years after the filing of the patent in *Magsil* for others to reach a 604% change in resistance, here, it took scientists at Chugai almost 10 years to discover the accused product emicizumab, which increases procoagulant activity by 10%. *See supra* § VI ¶ 28; Kitazawa Dep. Tr. at 241:12–20. The highest amount that any antibody disclosed in the specification is estimated to have increased the procoagulant activity of Factor IXa is by 3.75% of normal Factor VIII levels. *See supra* § VI ¶ 4. Both are far short of normal Factor VIII levels (at least 40%). *See id.* Although agreeing that an antibody that increases procoagulant activity by the same amount as would a normal level of Factor VIII would be within the scope of the claims, *see* Marasco Dep. Tr. at 236:12–18, Baxalta's expert concedes "it would be difficult or indeed impossible to create" an antibody that increases the procoagulant activity by such an amount, Krishnaswamy Dep. Tr. at 212:18–213:7. A specification cannot adequately enable something that is admittedly impossible to accomplish. *See, e.g.*, *Tr. of Boston Univ. v. Everlight Elecs. Co.*, 896 F.3d 1357, 1362 (Fed. Cir. 2018) ("We can safely conclude that the specification does not enable what the experts agree is physically impossible."). But even as to the compounds within the realm of possibility, there is no enablement.

The stated object of the invention is "to provide a preparation for the treatment of blood coagulation disorders . . . ." '590 patent, col. 2, ll. 25–28; *see also id.* at col. 9, ll. 25–36 ("The

present antibodies . . . are suitable for therapeutic use . . . ."). And the primary utility disclosed in the '590 patent for antibodies and antibody fragments that "increase[] the procoagulant activity of Factor IXa" is a therapeutic one. *See id.* col. 1, ll. 32–35; *id.*, col. 2, ll. 22–33; *id.*, col. 2, ll. 39–44; *id.*, col. 9, ll. 25–36; *id.*, col. 9, ll. 50–61.[17] But as discussed, the highest amount by which any antibody disclosed in the specification is estimated to have increased the procoagulant activity of Factor IXa (3.75%), would only be capable of moving a patient with hemophilia A classified as severe to a moderate classification. *See supra* § VI ¶ 4. It would not be capable of moving a patient with severe hemophilia to a mild classification. Although the experts debate whether such a small amount of procoagulant activity could be therapeutically useful for some patients, *see, e.g.*, Krishnaswamy Rpt. ¶¶ 115–118; Sheehan Rebuttal Rpt. ¶¶ 40–63, Baxalta acknowledges in the 20 years since the '590 patent was filed, it has never brought to market a product embodying the '590 patent's invention, *see* Scheiflinger Dep. Tr. 48:25–49:11 ("no company has ever brought to market a monospecific antibody to Factor IXa to treat hemophilia"); *see also* Hr'g Tr. at 55:09–12 (counsel for Baxalta agreeing that none of the eleven examples in the patent were "developed into a therapeutic product").

---

[17] After discussing the therapeutic utility of the claimed subject matter, the specification proposes other uses for the disclosed antibodies and antibody fragments, which only require that they bind to factor IX/IXa (not increase the procoagulant activity of factor IXa):

> Moreover, the antibodies and antibody derivatives according to the invention may also be used for industrial applications, e.g. for the purification of factor IX/factor IXa by means of affinity chromatography, or as a component of detection methods (e.g. ELISA assays), or as an agent for identification of and interaction with functional domains of a target protein.

'590 patent, col. 9, ll. 50–56. Even if true that a stated purpose of the patent was the use of antibodies in industrial applications, that can hardly be used to show the enablement of what is clearly the primary purpose of the patent and indisputably covered by the claims—the treatment of hemophilia A.

What is more, the patent also claims therapeutic effectiveness in the presence of Factor VIII inhibitors (claim 2), necessary for the treatment of inhibitor patients. The only antibody that was subjected to the aPTT and measured in the presence of Factor VIII inhibitors was 193/AD3, *see supra* § VI ¶ 25, for which Baxalta's expert concedes the specification lacks "[]sufficient information to accurately estimate the relevant rates of Factor Xa generation" in the chromogenic assay, Krishnaswamy Rpt. ¶ 122. Genentech's expert estimated, based on Figure 6A in the patent, that 193/AD3 (the only antibody tested in the presence of Factor VIII inhibitors) increases the level of procoagulant activity only at about 0.3–0.4% equivalent of Factor VIII activity, a marginal amount. *See supra* § VI ¶ 6.

Baxalta's expert now concedes that the patent's assertions that antibodies of the invention have therapeutic utility was merely "aspirational," Krishnaswamy Dep. Tr. at 42:05–45:14, and agrees that there is "not enough information conveyed in the patent to tell whether an antibody such as [198/A1] would have activity sufficient for clinical use," *id.* at 185:23–186:05.

Baxalta's only response to *MagSil* is that it is "factually inapposite" because it pertains only to claims to "a marginal improvement to a known quality," Pl.'s Opp'n at 34, and that a lower enablement standard should somehow apply to claims covering subject matters with previously unknown qualities, as Baxalta contends is the case here, *id.*; *see also* Hr'g Tr. 57:20–58:1–2.[18] Baxalta cites no caselaw for such a proposition, and the court does not read *MagSil* to be cabined in such a way. In *Plant Genetic Systems*, the Federal Circuit made clear that the enablement requirement is the same regardless of whether marginal or major advances are the subject of a

[18] "[The court]: There's no upper limit? [Counsel for Baxalta]: No, Your Honor, . . . this is not a quality that was known in advance."

patent. 315 F.3d at 1339–40 (rejecting as "not supported by precedents" an argument that a patent was entitled to a lower enablement requirement for a "pioneering" patent).

That the '590 patent discloses a starting point for further research by disclosing a monospecific murine antibody that binds to Factor IX or IXa and increases the procoagulant activity of Factor IXa by a small amount is not sufficient enablement. *See Wyeth*, 720 F.3d at 1386 (determining that the specification provided "only a starting point for further iterative research in an unpredictable and poorly understood field"); *Storer v. Clark*, 860 F.3d 1340, 1350 (Fed. Cir. 2017) ("The specification need not recite textbook science, but it must be more than an invitation for further research.").

C. Structural Scope

Third, it is established that a claim is not enabled if it is structurally broad and there are insufficient working examples and guidance to enable the full scope of the structural limitations. *See, e.g.*, *Idenix*, 941 F.3d at 1157–58. Here, claims 2–4 and 19–20 are necessarily within the scope of claim 1 because they are dependent claims. It is undisputed that the '590 patent provides no working examples of two of the four *Markush*-group members in claims 3 and 20 (IgE and IgA), nor does the specification provide any guidance as to how one skilled in the art would alter the process disclosed in the patent or engineer antibodies to arrive at those isotypes. In fact, Baxalta's expert testified that it would be exceedingly rare to discover antibodies of those isotypes for which there are no working examples. *See supra* § VI ¶ 3(a). Although the specification states that a class switch "may also be caused in a directed manner by means of genetic engineering methods" known in the prior art, '590 patent, col. 6, ll. 41–45, the inventors of the '590 patent did not perform such engineering or provide any specific guidance beyond reference to what was known in the prior art.

Nor are there any working examples of seven of the nine members of the *Markush* group in claim 4—"a chimeric antibody, a humanized antibody, . . . , a bispecific antibody, a diabody, and di-, oligo- or multimers thereof." *See supra* § VI ¶ 3(b). Although a POSITA would have a general understanding of the process for modifying an antibody into these various formats, there is no specific direction as to the structure (*e.g.*, to what antigen the second arm of a bispecific antibody should bind),[19] and no assurance that, once the modifications are made, the antibody will retain the same functional qualities much less that making it bispecific would enhance its properties. *See id.* ¶ 26.

Of particular significance is the absence of any working examples of a humanized antibody (claim 19). While there are references to humanized antibodies in the list of antibody types known in the art, '590 patent col. 6, ll. 15–19, 49–63, and a reference to prior-art humanization techniques, *see id.* at col. 7, l. 66–col. 8, l.4, the inventors never created a humanized antibody, provided no guidance in the specification as to how to create a humanized antibody that would exhibit the claimed function, and never determined whether humanizing an antibody (that would otherwise satisfy the claim limitations) would preserve its claimed procoagulant function, *see supra* § VI ¶ 27*;* Marasco Dep. Tr. at 120:13–20, 125:24–126:04. And because there is no way to predict whether, after humanization, an antibody or antibody fragment will retain its ability to bind to factor IX/IXa and increase the procoagulant activity of factor IXa, additional screening would be

[19] Although Baxalta's expert opines that "a POSITA would know to identify a second binding specificity for the bispecific form of the claimed invention," Pl.'s Opp'n, Ex. 12, ECF No. 424-13, Chang Rpt. ¶ 81, and that a "natural choice for a second binding specificity would be one of the two proteins associated with Factor IXa in the coagulation cascade: Factor VIII or Factor X," *id.* at ¶ 82, an inventor of the '590 patent admitted that, at the time of filing, they had not thought of or disclosed which antigens a bispecific antibody would bind, *see* Kerschbaumer Dep. Tr. at 19:459–461, and another Baxalta expert conceded that additional confirmatory testing would be necessary following modification of an antibody into a different format to ensure that the binding and activating functions of the antibody remained in place, Marasco Dep. Tr. at 127:24–128:25.

necessary after modification of the antibody. *See supra* § VI ¶ 27; Garcia Rpt. ¶¶ 84–85, 167. Baxalta's expert Dr. Marasco wrote in a patent of his own, and confirmed at his deposition, that "humanizing an antibody is not as efficient a process as sometimes presented," and that after humanization an antibody "frequently does not have the same effectiveness as the original murine antibody." Marasco Dep. Tr. at 130:02–131:07.

D. Dependent Claims

So far, the court has been focused on claim 1, but the dependent claims fare no better. If anything, it is even clearer that the dependent claims are invalid given the dearth of working examples for the vast majority of what they claim as a matter of structure, as discussed *supra* § VIII.C. And the functional limitations of claim 1 apply equally to the dependent claims because they each claim the antibody or antibody fragment according to claim 1 and do not narrow the functional limitations of claim 1 in any way. *Alcon Rsch., Ltd. v. Apotex Inc.*, 687 F.3d 1362, 1367 (Fed. Cir. 2012) ("[B]ecause a dependent claim narrows the claim from which it depends, it must 'incorporate . . . all the limitations of the claim to which it refers.'" (quotation omitted)). Accordingly, given the limited working examples for the structural limitations of the asserted dependent claims—including no working examples of antibodies that satisfy claim 19 (humanized antibodies), only examples of two of the four isotypes listed in claims 3 and 20, and no working examples of seven of the nine structural formats listed in claim 4—combined with the broad scope of the functional limitations under claim 1 and the lack of guidance in the specification, no reasonable jury could find the dependent claims are enabled.

E. Nonenablement of Emicizumab

Finally, it is significant that the patent does not remotely enable the accused antibody, emicizumab, which must fall within the scope of the claims to establish an infringement claim.

Emicizumab increases the procoagulant effect by approximately 10%[20]—an amount that has proven capable of reducing bleeding episodes in inhibitor patients by a clinically significant amount. *See* Smith Decl. ¶¶ 44, 49; Malackowski Rpt. at 34, 36 . Yet, as discussed, the patent does not disclose an antibody that has procoagulant activity anywhere near that amount. None of the 11 disclosed antibodies in the specification increase the procoagulant activity of factor IXa more than 3.75%, and it is unknown whether that example would perform the same in the presence of Factor VIII inhibitors. *See supra* § VI ¶¶ 4, 6.

Moreover, key to emicizumab's therapeutic effectiveness is its structure as a bispecific humanized antibody, *see* Sampei Article at 2, and as discussed, there is no working example of either a bispecific or humanized antibody in the specification of the '590 patent, let alone an antibody that is both. Two inventors of the '590 patent, Scheiflinger and Kerschbaumer, admitted they did not make a bispecific antibody, *see* Scheiflinger Dep. Tr. at 68:08–19; Kerschbaumer Tr. at 18:429–434, and Baxalta's expert, Dr. Krishnaswamy, conceded that the patent's "language" about antibodies of the invention have therapeutic utility was merely "aspirational," Krishnaswamy Dep. Tr. at 42:05–45:14. Significantly, it took Chugai over ten years of multi-phased experimentation and the screening of tens of thousands of candidate compounds to discover emicizumab. *See supra* § VI ¶ 28.

Although Baxalta concedes that emicizumab is within the scope of the claims, it now argues that the court should not be concerned with the lack of enablement of emicizumab because

---

[20] Baxalta's expert disputes the accuracy of this 10 percent figure, *see* Krishnaswamy Rpt ¶ 112, but that opinion is based on testing of emicizumab at a diluted concentration level and says nothing of the concentration level used in the treatment of hemophilia A. Moreover, the literature relied on by the same expert states that emicizumab at the treatment concentration level is "assumed to be equivalent to that of . . . 10% FVIII." Uchida et al*., A first-in-human phase 1 study of ACE910, a novel factor VIII–mimetic bispecific antibody, in healthy subjects*, Blood 127(13):1633–1641 (2016) (cited as Ex. E in Krishnaswamy Rpt).

the high coagulant effect of emicizumab may be due to the fact that it is bispecific and binds Factor X with the other arm, whereas the asserted claims involve the arm that binds Factor IX. *See* Hr'g Tr. at 50:20–51:05. In that event, Baxalta suggests maybe a compound is not within the scope of the claims if the procoagulant effect is only caused by a bispecific antibody's arm that binds to Factor X. *See id.* at 52:03–19.[21] This convoluted argument, offered for the first time at the Summary Judgment hearing, does nothing to show enablement. Baxalta convinced the Federal Circuit that bispecific antibodies are within the scope of the claims. It cannot now prevail by arguing that one bispecific antibody is perhaps not within the scope of the claims if its procoagulant activity results from its bispecific nature.

Past decisions have advised that patents should be awarded to the true inventor and that the enablement requirement serves an important purpose in this respect. *See Amgen Inc. v. Sanofi, Aventisub LLC*, 850 F. App'x 794, 796 (Fed. Cir. 2021) (denying rehearing en banc) ("One should not gain exclusivity over claimed subject matter without disclosing how to make and use it. And if one considers that one has invented a group of compositions defined by a genus but does not know enough to fully enable that genus, one would suppress innovation if one were able to claim such a broad genus, not enhance it."); *see also J.E.M. Ag Supply*, 534 U.S. at 142 (identifying an enabling disclosure as the "*quid pro quo* of the right to exclude" (quoting *Kewanee Oil Co. v.*

---

[21] Counsel: "The claimed invention is about the bispecific antibody with the arm that binds Factor 9, 9A and that arm that exhibits the procoagulant activity on Factor 9, 9A, and it's not necessary to enable or to fully describe even the portions of the . . . composition that are not part of the claim."

Court: "[S]o there would be no infringement here if Hemlibra procoagulant activity resulted from the Factor 10 binding and not from the fact that it binds, the other chain binds to Factor 9A?"

Counsel: "If the only procoagulant activity [] came from the binding of Factor 10 and there was no procoagulant activity as a result of the Factor [IX/IXA] arm, my understanding is that there would be no infringement."

Bicron Corp., 416 U.S. 470, 484 (1974)); *McRO*, 959 F.3d at 1099–100 ("The requirement of enablement . . . enforces the essential '*quid pro quo* of the patent bargain.'" (quoting *AK Steel Corp. v. Sollac*, 344 F.3d 1234, 1244 (Fed. Cir. 2003)).

That is, the enablement requirement ensures that the entity that does the hard work to invent a useful compound is the recipient of the patent, not some earlier inventor who may have conceived of such a therapy or made the first step in research, but did not enable its ultimate production. *See Genentech*, 108 F.3d at 1366 ("Patent protection is granted in return for an enabling disclosure of an invention, not for vague intimations of general ideas that may or may not be workable."); *see also Wyeth*, 720 F.3d at 1386 (finding that disclosing "only a starting point for further iterative research in an unpredictable and poorly understood field" does not constitute sufficient enablement). That is the situation here. The court cannot allow Baxalta to provide a starting point for further research and then claim "someone else's solution to the problem." *Genentech*, 108 F.3d at 1366.

CONCLUSION

For the foregoing reasons, the court GRANTS Genentech's motion for summary judgment of invalidity for lack of enablement and DENIES as moot Genentech's motion for summary judgment in all other respects.

Honorable Timothy B. Dyk
United States Circuit Judge, sitting by designation

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

BAXALTA INCORPORATED and BAXALTA GMBH,

Plaintiffs,

v.

GENENTECH, INC. and CHUGAI PHARMACEUTICAL CO., LTD.,

Defendants.

Civil Action No. 17-509-TBD

ORDER

For reasons discussed in the accompanying Memorandum Opinion issued this 13th day of January, 2022,

IT IS HEREBY ORDERED that:

1. Genentech's Motion for Summary Judgment, ECF No. 407, is **GRANTED** on the ground that the asserted claims of the '590 patent are invalid for lack of enablement, and judgment is entered in favor of Genentech.

2. As the Memorandum Opinion was filed under seal, the parties shall meet and confer and shall on or before January 18, 2022, submit any proposed redactions. The parties are advised that redactions are strongly disfavored and proposed redactions must be accompanied by a showing of good cause. Thereafter, the court will issue a public version of its Memorandum Opinion.

This is a final appealable order.

SO ORDERED.

Honorable Timothy B. Dyk
United States Circuit Judge, sitting by designation

US007033590B1

(12) **United States Patent**
Scheiflinger et al.

(10) **Patent No.: US 7,033,590 B1**
(45) **Date of Patent: Apr. 25, 2006**

(54) **FACTOR IX/FACTOR IXA ACTIVATING ANTIBODIES AND ANTIBODY DERIVATIVES**

(75) Inventors: **Friedrich Scheiflinger**, Vienna (AT); **Randolf Kerschbaumer**, Vienna (AT); **Falko-Guenter Falkner**, Orth/Donau (AT); **Friedrich Dorner**, Vienna (AT); **Hans-Peter Schwarz**, Vienna (AT)

(73) Assignee: **Baxter Aktiengesellschaft**, Vienna (AT)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 462 days.

(21) Appl. No.: **09/661,992**

(22) Filed: **Sep. 14, 2000**

(30) **Foreign Application Priority Data**

Sep. 14, 1999 (AT) 1576/99

(51) **Int. Cl.**

A61K 39/395	(2006.01)
A61K 38/04	(2006.01)
C12N 5/20	(2006.01)
C07K 16/00	(2006.01)
C07K 16/34	(2006.01)

(52) **U.S. Cl.** **424/145.1**; 435/326; 530/388.25; 530/387.1; 530/327; 530/328; 530/389.3

(58) **Field of Classification Search** 530/387.3, 530/388.25, 389.3, 327, 328; 424/133.1, 424/145.1; 435/326
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,395,396	A		7/1983	Eibl et al.
4,873,316	A		10/1989	Meade et al.
5,932,706	A		8/1999	Mertens et al.
6,391,299	B1	*	5/2002	Blackburn et al.
6,632,927	B1	*	10/2003	Adair et al.

FOREIGN PATENT DOCUMENTS

WO	WO95/13300	5/1995
WO	WO97/26010	7/1997
WO	WO99/01476	1/1999

OTHER PUBLICATIONS

Panka et al. Variable region framework differences result in decreased or increased affinity of variant anti–digoxin antibodies. Proc Natl Acad Sci U S A. 85(9):3080–3084, 1988.*

Rudikoff et al. Single amino acid substitution altering antigen–binding specificity. Proc Natl Acad Sci U S A. 79(6):1979–1983, 1982.*

Ames, R.S. et al., *Conversion of Murina Fabs Isolated From a Combinatorial Phage Display Library to Full Length Immunoglobulins, J. Immunol. Methods*, pp. 177–186 (1995).

Bajaj, S.P. et al., *A Monoclonal Antibody to Factor IX That Inhibits the Factor VIII:Ca Potentiation of Factor X Activation, The Journal of Biological Chemistry*, 260(21), pp. 11574–11580 (1985).

Bessos, H., et al., *The Characterization of a Panel of Monoclonal Antibodies to Human Coagulation Factor IX, Thrombosis Research,* 40, pp. 863–867 (1985).

Cao, Y. et al., *Bispecific Antibodies as Novel Bioconjugates, Bioconjugate Chemistry*, 9(6); pp. 635–644 (1998).

Cohen, F.E., et al., *The Combinatorial Approach, Protein Structure Prediction—A Practical Approach* (Ed. M.J.E. Sternberg), Oxford University Press, Ch. 9, pp. 207–227 (1996).

Engelhardt, O., et al., *Two Step Cloning of Antibody Variable Domains in a Phage Display Vector, Biotechniques*, 17, p. 44–46 (1994).

Esser, C., et al., *Immunoglobulin Class Switching: Molecular and Cellular Analysis, Annu. Rev. Immunol.*, 8, p. 717–735 (1990).

Evan, G.I., et al., *Isolation of Monoclonal Antibodies Specific for Human c–myc Proto–Oncogene Product, Mol. Cell. Biol.*, 5(12), p. 3610–3616 (1985).

Fay, P.J., et al., *Factor Villa A2 Subunit Residues 558–565 Represent a Factor IXa Interactive Site, Journal of Biological Chemistry*, 269(32), p. 20522–20527 (1994).

Frazier, D., et al., *Mapping of Monoclonal Antibodies to Human Factor IX, Blood*, 74(3), p. 971–977 (1989).

Gao, C., et al., *Making Artifical Antibodies: A Format for Phage Display of Combinatorial Heterodimeric Arrays, Proc. Natl. Acad. Sci.*, 96, p. 6025–6030 (1999).

Grassy, G., et al., *Computer–Assisted Rational Design of Immunosuppressive Compounds, Nature Biotechnology*, 16, p. 748–752 (1998).

Greer, J., et al., *Application of the Three–Dimensional Structures of Protein Target Molecules in Structure–Based Drug Design, Journal of Medicinal Chemistry*, 37(8), p. 1035–1054 (1994).

Harlow, E., et al., *2. Antibody Molecules, Antibodies—A Laboratory Manual*; pp. 7–22 (1988).

Harlow, E., et al., *3. Antibody–Antigen Interactions, Antibodies—A Laboratory Manual*; p. 23–35 (1988).

Harlow, E., et al., *6. Monoclonal Antibodies, Antibodies—A Laboratory Manual*; p. 139–243 (1988).

Hochuli, E., et al., *Genetic Approach to Facilitate Purfication of Recombinant Proteins with a Novel Metal Chelate Adsorbent, Biotechnology*, 6, p. 1321–1325 (1988).

Huston, J.S., et al., *Medical Applications of Single–Chain Antibodies, Intern. Rev. Immunol.*, 10, p. 195–217 (1993).

(Continued)

Primary Examiner—Christina Chan
Assistant Examiner—Maher Haddad
(74) *Attorney, Agent, or Firm*—Townsend and Townsend and Crew LLP

(57) **ABSTRACT**

An antibody or antibody derivative against factor IX/activated factor IX (FIXa) which increases the procoagulant activity of FIXa.

22 Claims, 61 Drawing Sheets

OTHER PUBLICATIONS

Jones, D.T., et al., *Protein Folds and Their Recognition from Sequence, Protein Structure Prediction—A Practive Approach* (Ed. M.J.E. Sternberg), Oxford University Press, Ch. 8, p. 174–206 (1996).

Jones, P.T., et al., *Replacing the Complementarity–Determining Regions in a Human Antibody with Those from a Mouse, Nature*, 321, p. 522–525 (1986).

Jorquera, J.I., et al., *Synthetic Peptides Derived from Residues 698 to 710 of Factor VIII Inhibit Factor IXa Activity, Circulation*, 86, Abstract No. 2725, p. I–685 (1992).

Karpen, M.E., et al., *Modelling Protein Conformation by Molecular Mechanics and Dynamics, Protein Structure Prediction—A Practical Approach* (Ed. M.J.E. Sternberg), Oxford University Press, Ch. 10, p. 229–261 (1996).

Kemp, D.S., *Peptidomimetics and the Template Approach to Nucleation of B–sheets and a–helices in Peptides, TIBTECH*8, p. 249–255 (1990).

Kerschbaumer, R.J., et al, *pDAP2: A Vector for Construction of Alkaline Phosphatase Fusion–Proteins, Immunotechnology*, 2, p. 145–150 (1996).

Kerschbaumer, R.J. et al., *Single–Chain Fv Fusion Proteins Suitables as Coating and Detecting Reagents in a Double Antibody Sandwich Enzyme–Linked Immunosorbent Assay, Analytical Biochemistry*, 249, p. 219–227 (1997).

Lane, R.D., *A Short–Duration Polyethylene Glycol Fusion Technique for Increasing Production of Monoclonal Antibody–Secreting Hybridomas, Journal of Immunological Methods*, 81, p. 223–227 (1985).

Lenting, P.J., et al., *The Sequence Glu^{1811}–Lys^{1818} of Human Blood Coagulation Factor VIII Comprises a Binding Site for Activated Factor IX, Journal of Biological Chemistry*, 271(4), p. 1935–1940 (1996).

Liles, D.K., et al, *The Factor VIII Peptide Consisting of Amino Acids 698 to 712 Enhances Factor IXa Cleavage of Factor X, Blood*, 90(1), Abstract No. 2054, p. 463a (1997).

Lin, H–F., et al, *A Coagulation Factor IX–Deficient Mouse Model for Human Hemorphilia B, Blood*, 90(10), p. 3962–3966 (1997).

Malik, P., et al., *Multiple Display of Foreign Peptide Epitopes on Filamentous Bacteriophage Virions, Phage Display of Peptides and Proteins* (Ed. B. K. Kay et al.), Academic Press, p. 127–139 (1996).

Mann, K.G., et al., *Surface–Dependent Reactions of the Vitamin K–Dependent Enzyme Complexes, Blood*, 76(1), p. 1–16 (1990).

Mikaelsson, M., et al., *Standardization of VIII:C Assays: A Manufactorer's View, Scandinavian Journal of Haematology* (Ed. Nilsson et al.), 33, p. 79–86 (1984).

Nilsson, I.M. et al., *Induction of Split Tolerance and Clinical Cure in High–Responding Hemophiliacs with Factor IX Antibodies, Proc. Natl. Acad. Sci. USA*, 83, p. 9169–9173 (1986).

Persic, L., et al., *An Integrated Vector System For The Eukaryotic Expression of Antibodies of Their Fragments After Selection From Phase Display Libraries, Genes*, p. 9–18 (1997).

Pluckthun, A., et al., *New Protein Engineering Approaches to Multivalent and Bispecific Antibody Fragments, Immunotechnology*, 3, p. 83–105 (1997).

Raag, R., et al., *Single–Chain Fvs, FASEB Journal*, 9(1), pp. 73–80 (1995).

Rees, A.R., et al., *Antibody Combining Sites: Structure and Prediction, Protein Structure Prediction—A Practical Approach* (Ed. M.J.E. Sternberg), Oxford University Press, Ch. 7, p. 141–172 (1996).

Roitt, I.M., et al., *Molecules which Recognize Antigen, Immunology*, 2nd Edition, p. 5.1–5.11 (1989).

Sadler, J.E., et al., *Hemophila A, Hemophilia B, and von Willebrand's Disease, The Molecular Basis of Blood Diseases* (Ed. G. Stamatoyannopoulos et al.), p. 575–630 (1987).

Vaughan, T.J., et al., *Human Antibodies By Design, Nature Biotechnology*, p. 535–539 (1998).

Winter, G., et al., *Making Antibodies by Phage Display Technology, Annu. Rev. Immunol.*, 12, p. 433–455 (1994).

Zhong, D., et al., *Some Human Inhibitor Antibodies Interface with Factor VIII Binding to Factor IX, Blood*, 92(1), p. 136–142 (1998).

* cited by examiner

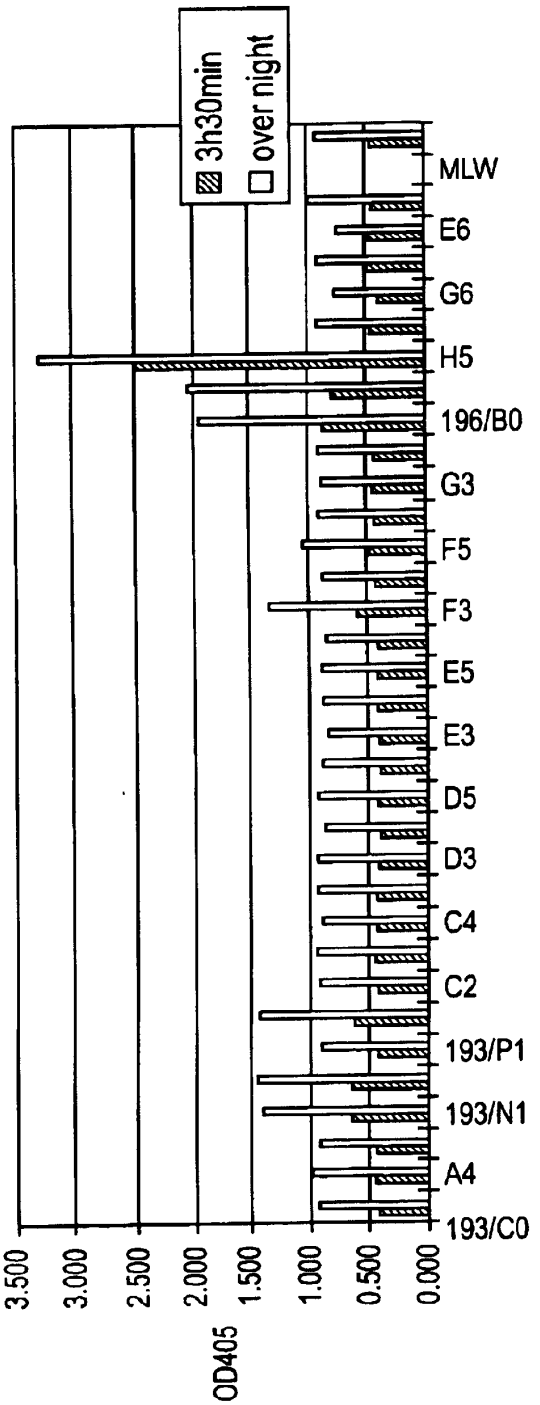

3h30min
over night
MLW
E6
G6
H5
196/B0
G3
F5
F3
E5
E3
D5
D3
C4
C2
193/P1
193/N1
A4
193/C0
clone #
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
OD405

FIG. 1

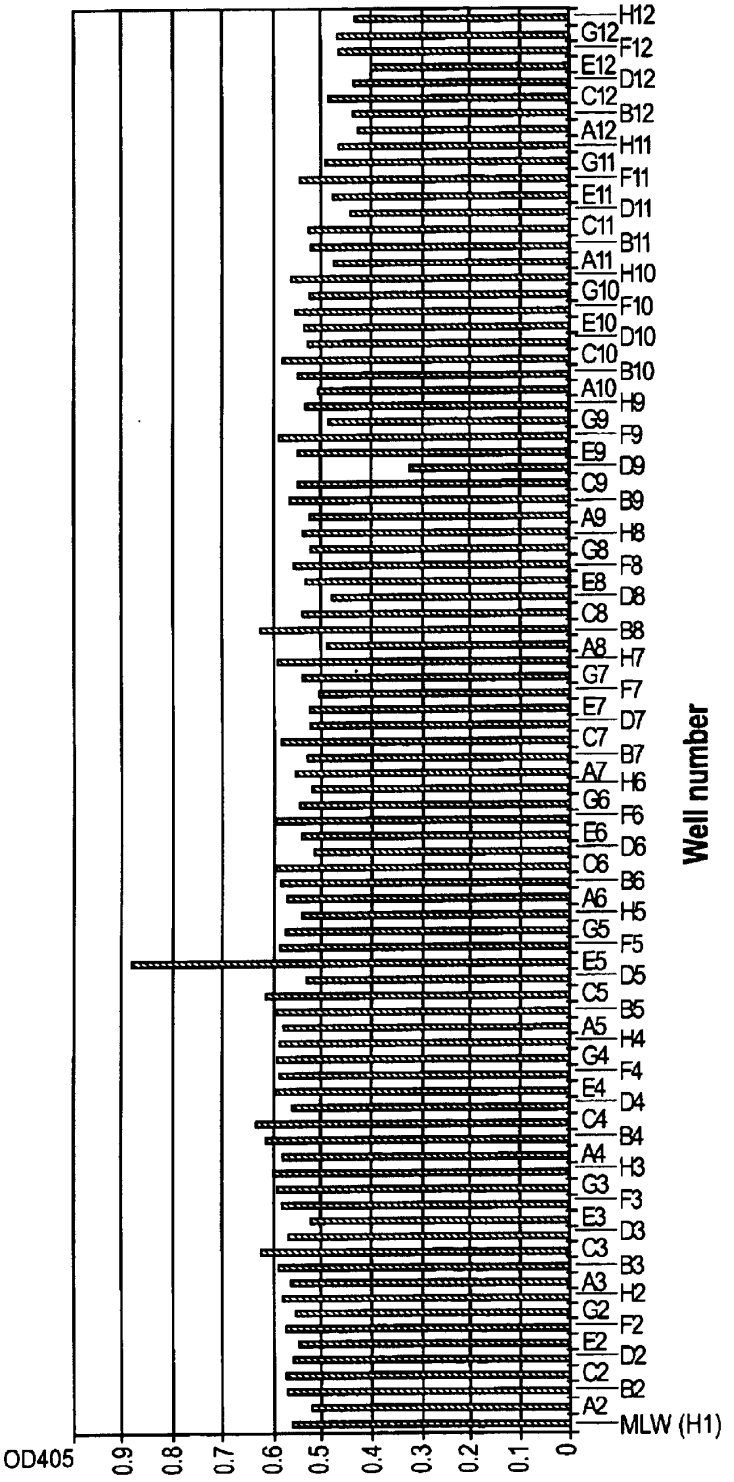

H12
G12
F12
E12
D12
C12
B12
A12
H11
G11
F11
E11
D11
C11
B11
A11
H10
G10
F10
E10
D10
C10
B10
A10
H9
G9
F9
E9
D9
C9
B9
A9
H8
G8
F8
E8
D8
C8
B8
A8
H7
G7
F7
E7
D7
C7
B7
A7
H6
G6
F6
E6
D6
C6
B6
A6
H5
G5
F5
E5
D5
C5
B5
A5
H4
G4
F4
E4
D4
C4
B4
A4
H3
G3
F3
E3
D3
C3
B3
A3
H2
G2
F2
E2
D2
C2
B2
A2
MLW (H1)
Well number
OD405
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0

FIG. 2

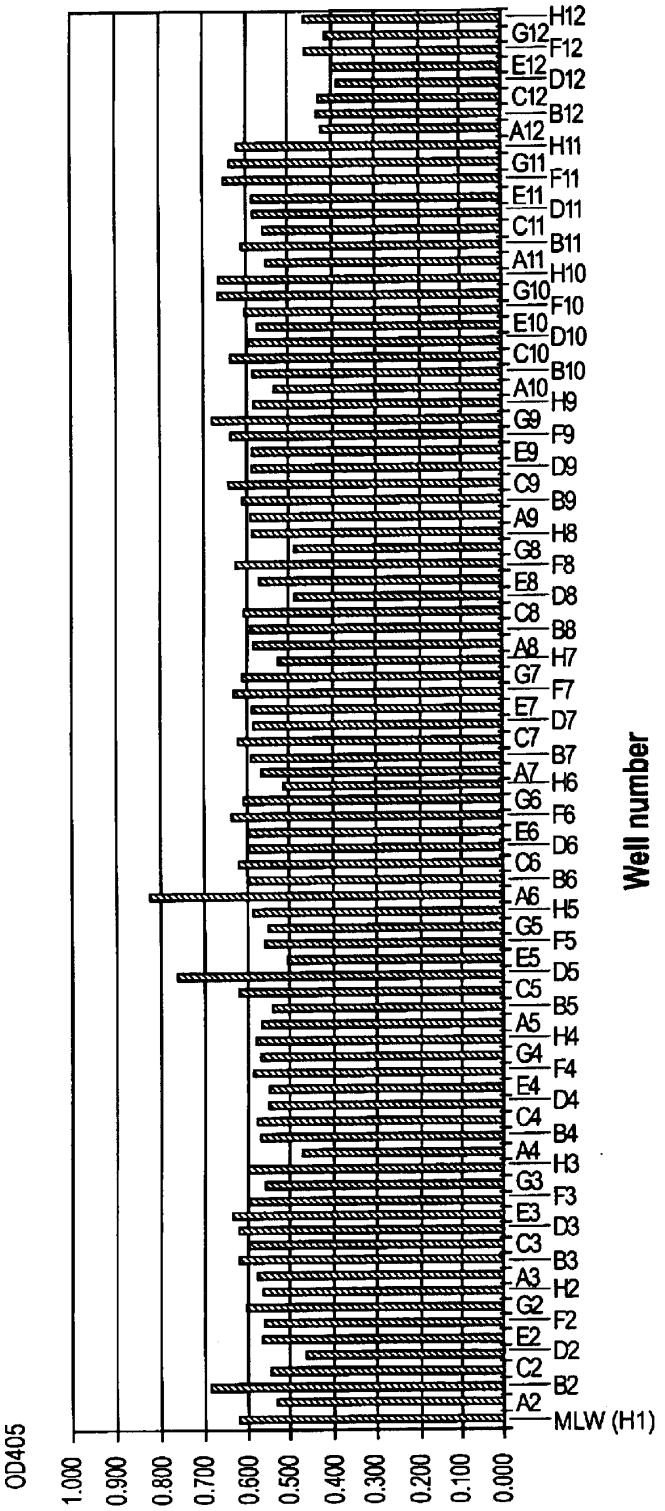

H12
G12
F12
E12
D12
C12
B12
A12
H11
G11
F11
E11
D11
C11
B11
A11
H10
G10
F10
E10
D10
C10
B10
A10
H9
G9
F9
E9
D9
C9
B9
A9
H8
G8
F8
E8
D8
C8
B8
A8
H7
G7
F7
E7
D7
C7
B7
A7
H6
G6
F6
E6
D6
C6
B6
A6
H5
G5
F5
E5
D5
C5
B5
A5
H4
G4
F4
E4
D4
C4
B4
A4
H3
G3
F3
E3
D3
C3
B3
A3
H2
G2
F2
E2
D2
C2
B2
A2
MLW (H1)
Well number
OD405
1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000

FIG. 3

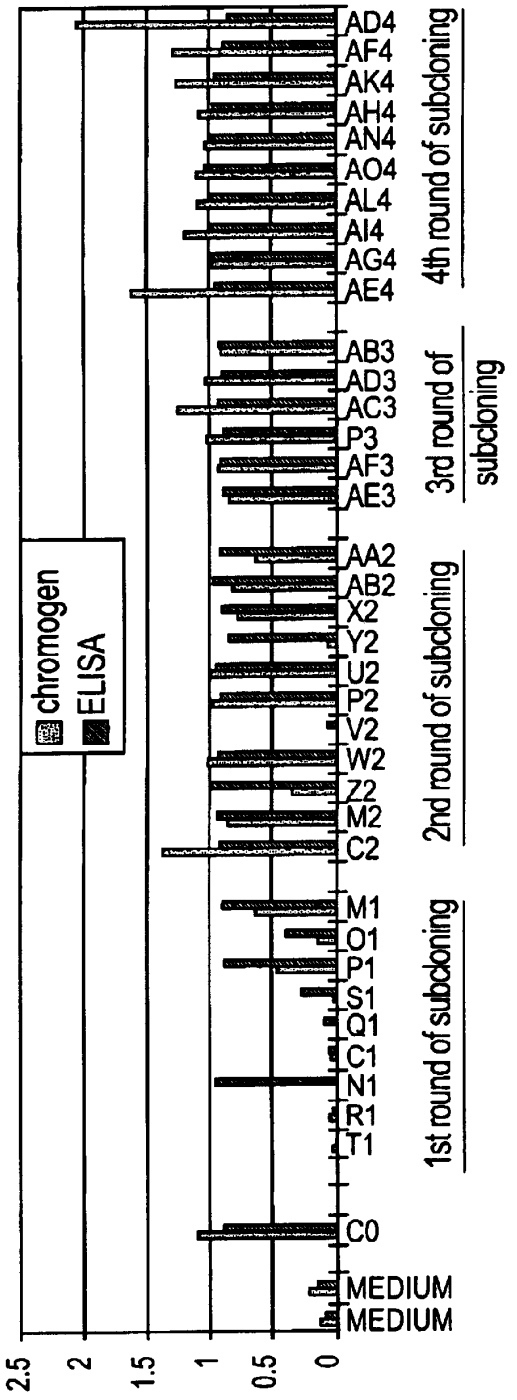

chromogen
ELISA
2.5
2
1.5
1
0.5
0
AD4
AF4
AK4
AH4
AN4
AO4
AL4
AI4
AG4
AE4
AB3
AD3
AC3
P3
AF3
AE3
AA2
AB2
X2
Y2
U2
P2
V2
W2
Z2
M2
C2
M1
O1
P1
S1
Q1
C1
N1
R1
T1
C0
MEDIUM
MEDIUM
4th round of subcloning
3rd round of subcloning
2nd round of subcloning
1st round of subcloning

FIG. 4

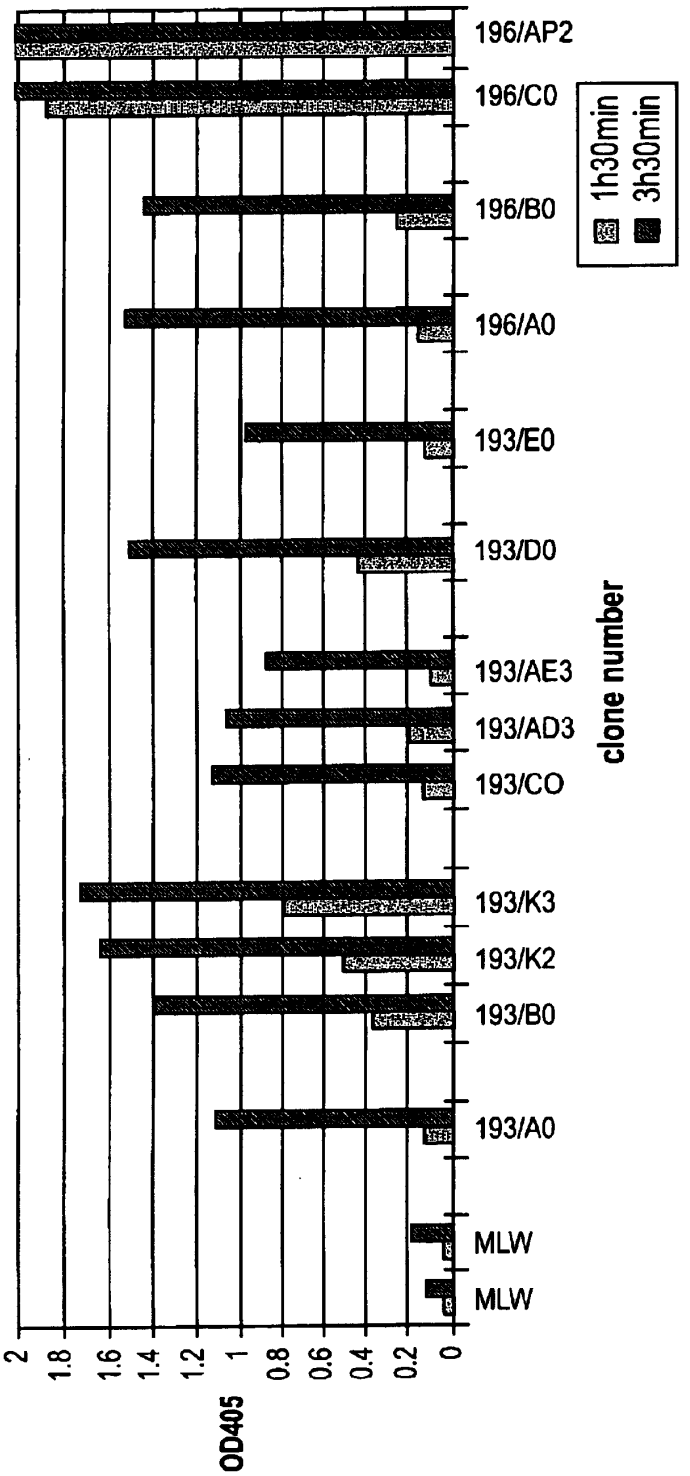

196/AP2
196/C0
196/B0
196/A0
193/E0
193/D0
193/AE3
193/AD3
193/CO
193/K3
193/K2
193/B0
193/A0
MLW
MLW
1h30min
3h30min
clone number
OD405
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0

FIG. 5

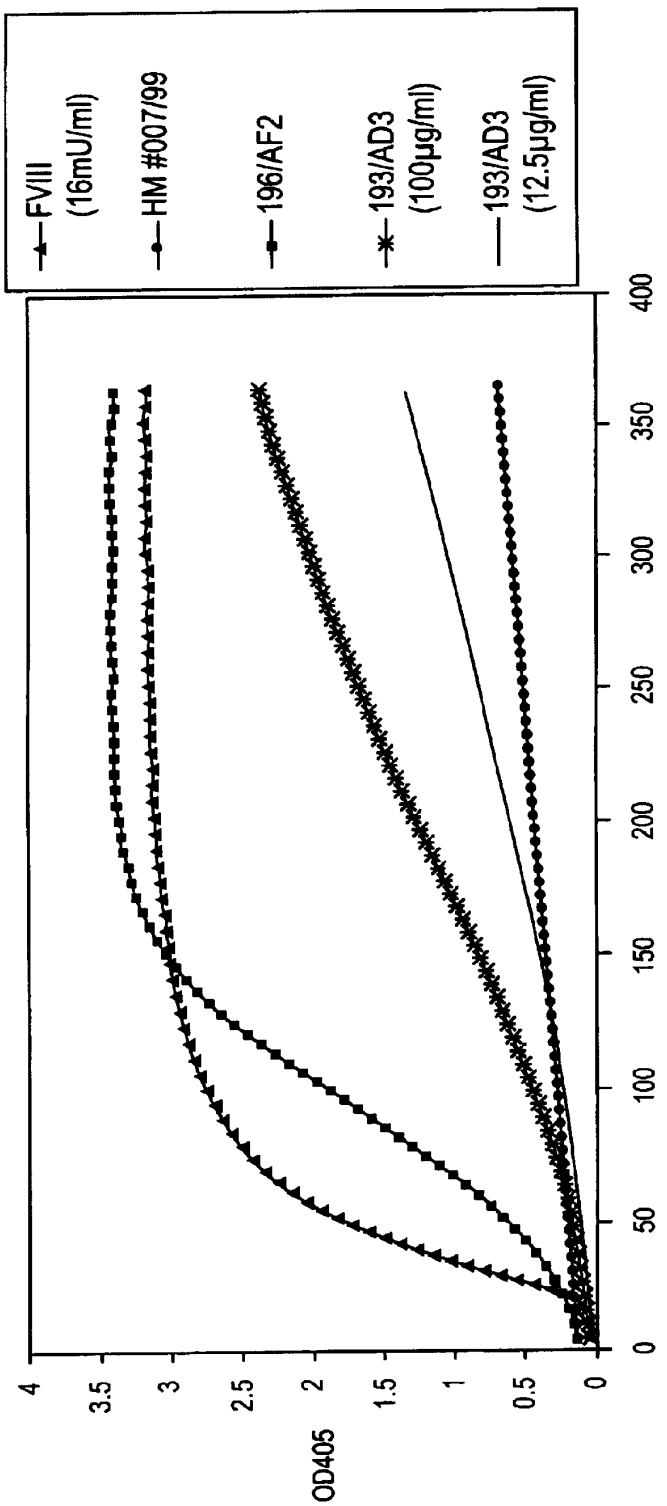

FVIII (16mU/ml)
HM #007/99
196/AF2
193/AD3 (100µg/ml)
193/AD3 (12.5µg/ml)
OD405
Time (min)
0
0.5
1
1.5
2
2.5
3
3.5
4
0
50
100
150
200
250
300
350
400

FIG. 6A

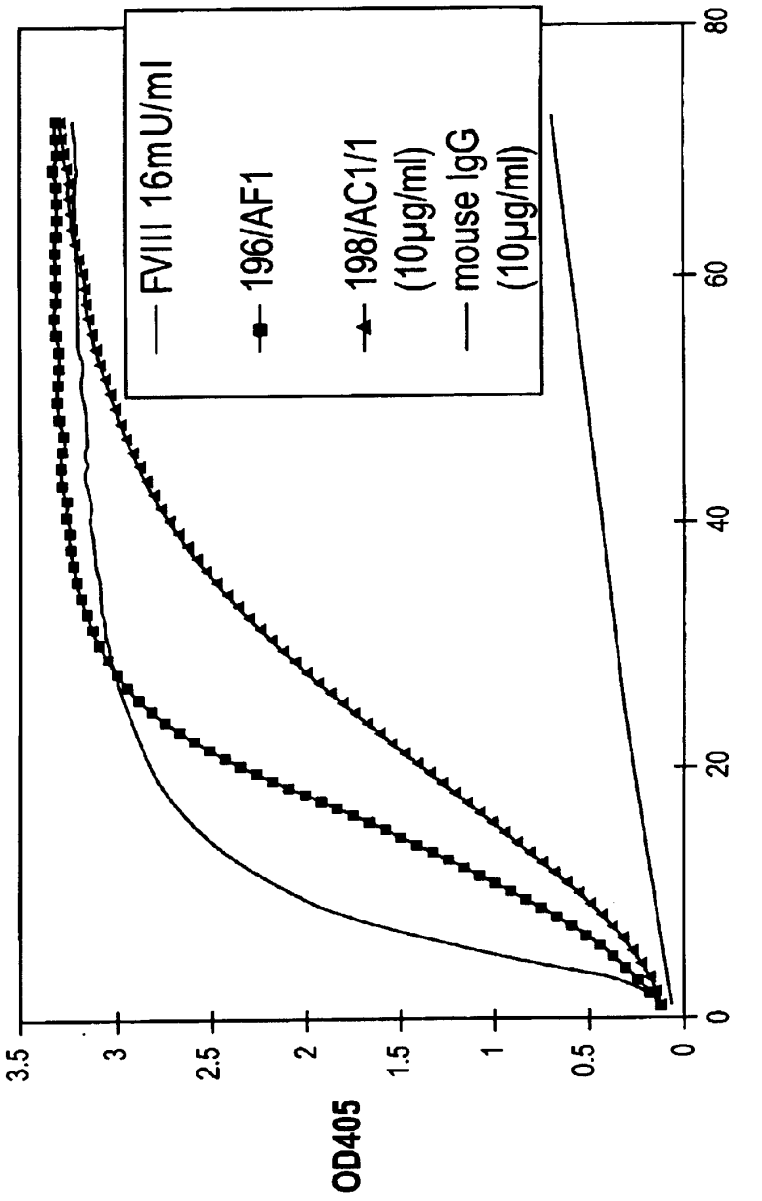

FVIII 16mU/ml
196/AF1
198/AC1/1 (10µg/ml)
mouse IgG (10µg/ml)
OD405
Scan Number
0
0.5
1
1.5
2
2.5
3
3.5
0
20
40
60
80

FIG. 6B

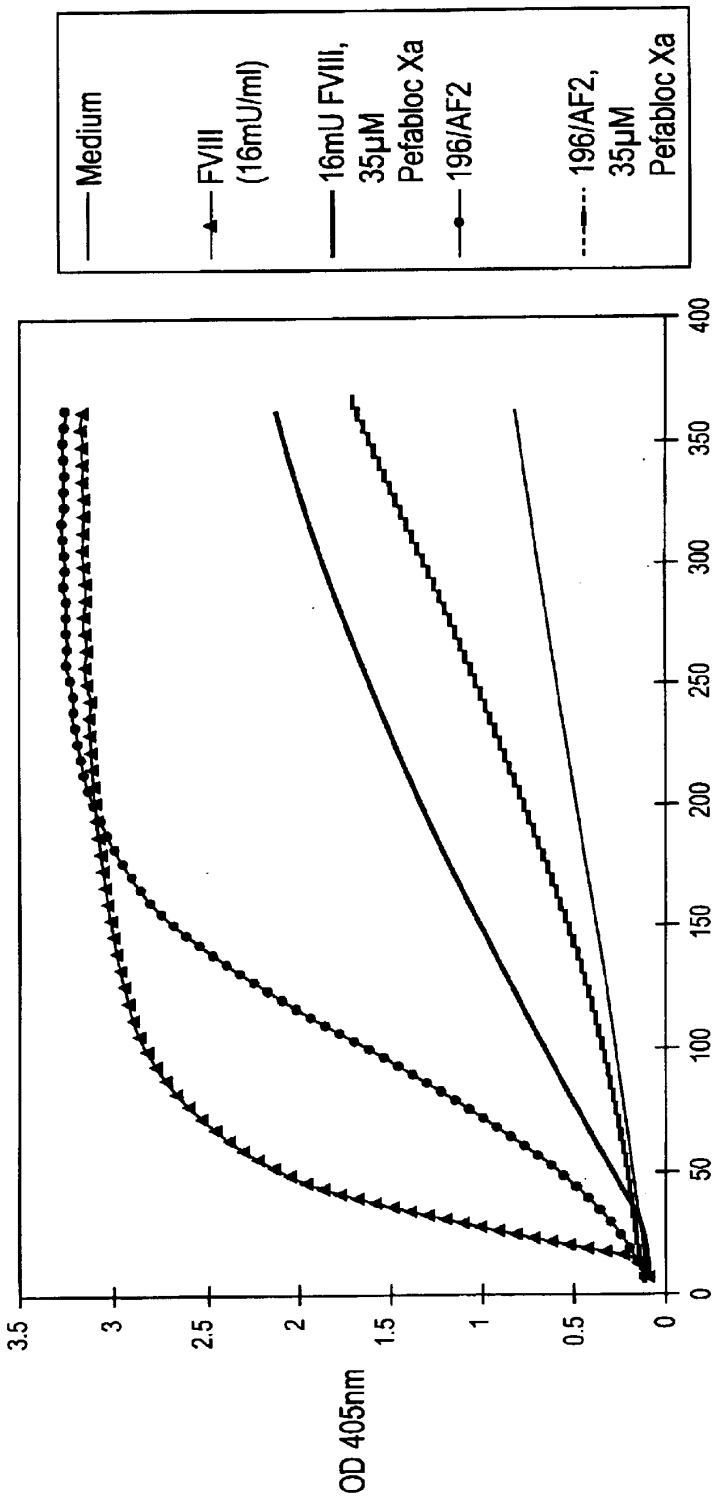

Medium
FVIII (16mU/ml)
16mU FVIII, 35µM Pefabloc Xa
196/AF2
196/AF2, 35µM Pefabloc Xa
OD 405nm
0
0.5
1
1.5
2
2.5
3
3.5
Time (min)
0
50
100
150
200
250
300
350
400

FIG. 7A

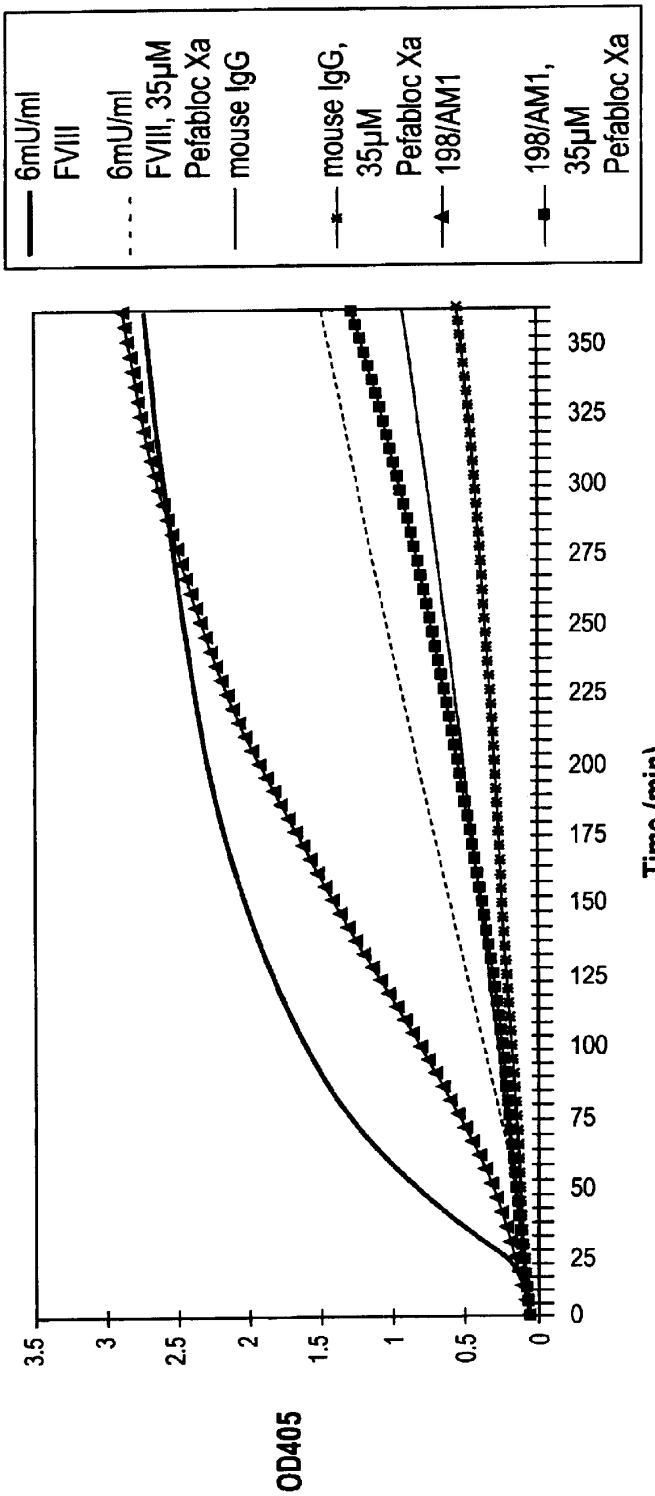

6mU/ml FVIII
6mU/ml FVIII, 35µM Pefabloc Xa
mouse IgG
mouse IgG, 35µM Pefabloc Xa
198/AM1
198/AM1, 35µM Pefabloc Xa
OD405
Time (min)
0
0.5
1
1.5
2
2.5
3
3.5
0
25
50
75
100
125
150
175
200
225
250
275
300
325
350

FIG. 7B

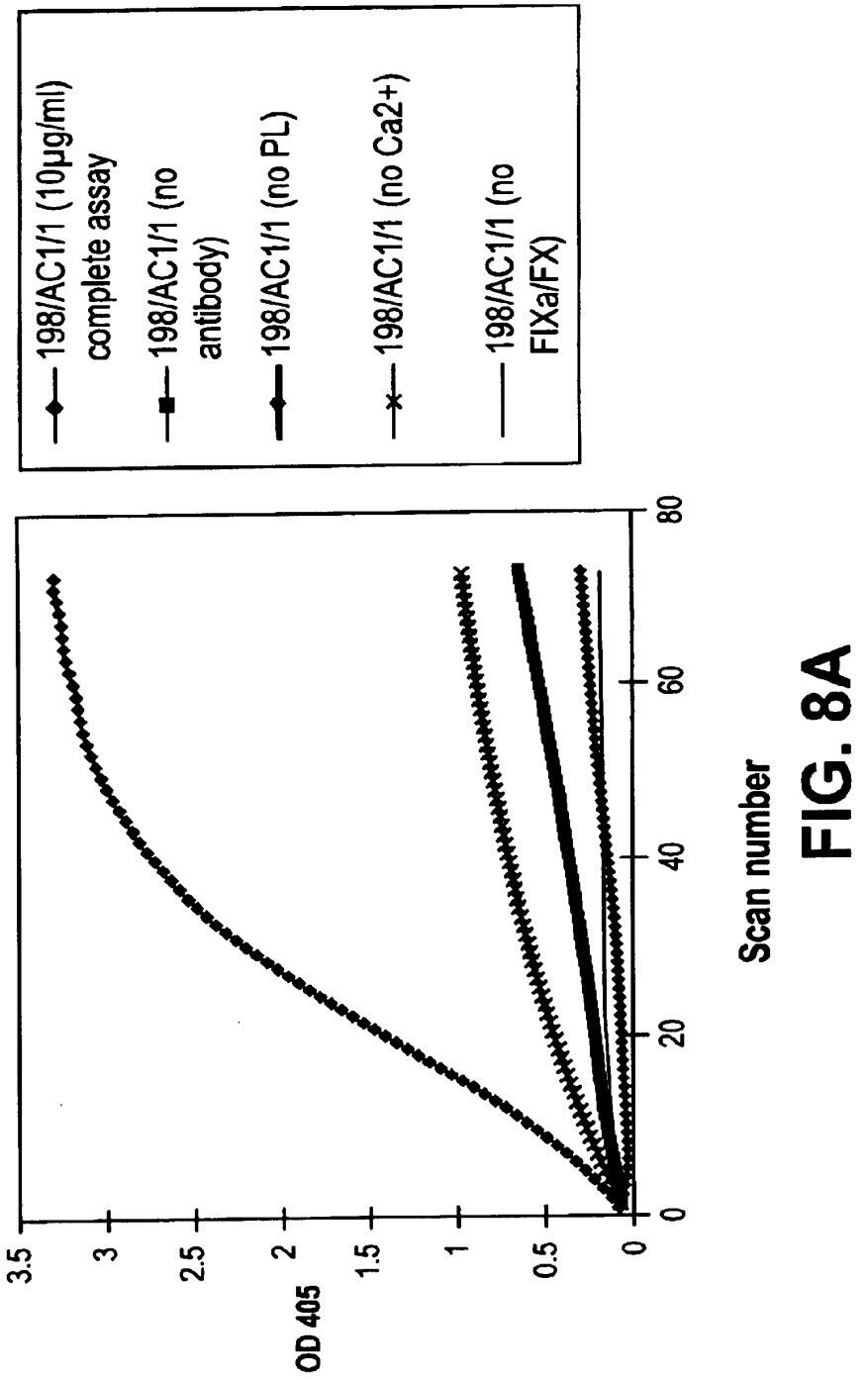

198/AC1/1 (10µg/ml) complete assay
198/AC1/1 (no antibody)
198/AC1/1 (no PL)
198/AC1/1 (no Ca2+)
198/AC1/1 (no FIXa/FX)
OD 405
3.5
3
2.5
2
1.5
1
0.5
0
0
20
40
60
80
Scan number

FIG. 8A

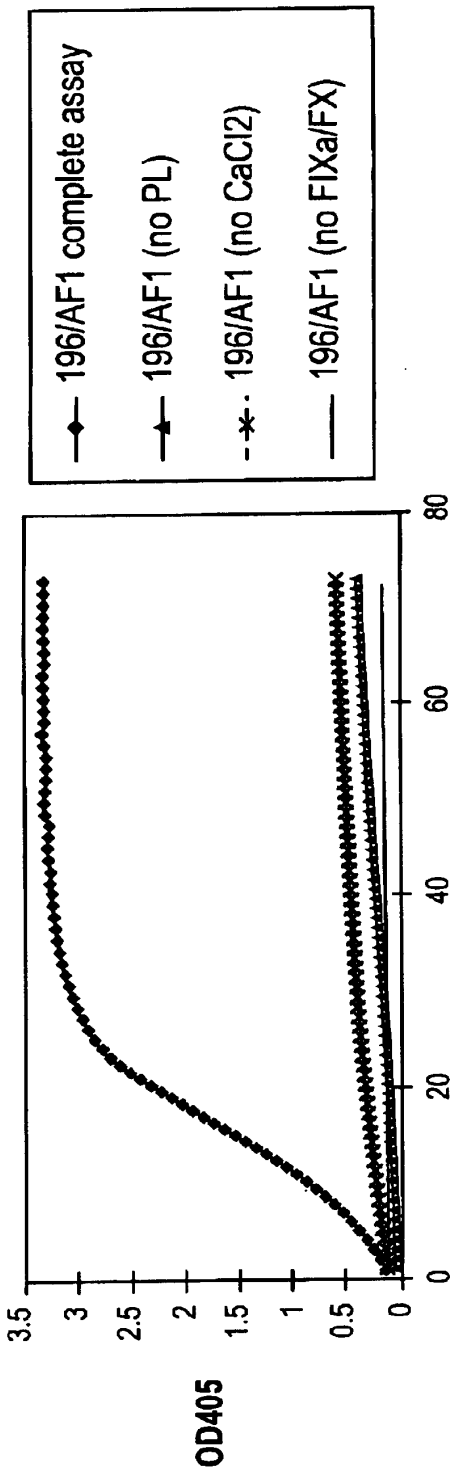

196/AF1 complete assay
196/AF1 (no PL)
196/AF1 (no CaCl2)
196/AF1 (no FIXa/FX)
OD405
0
0.5
1
1.5
2
2.5
3
3.5
0
20
40
60
80
scan number

FIG. 8B

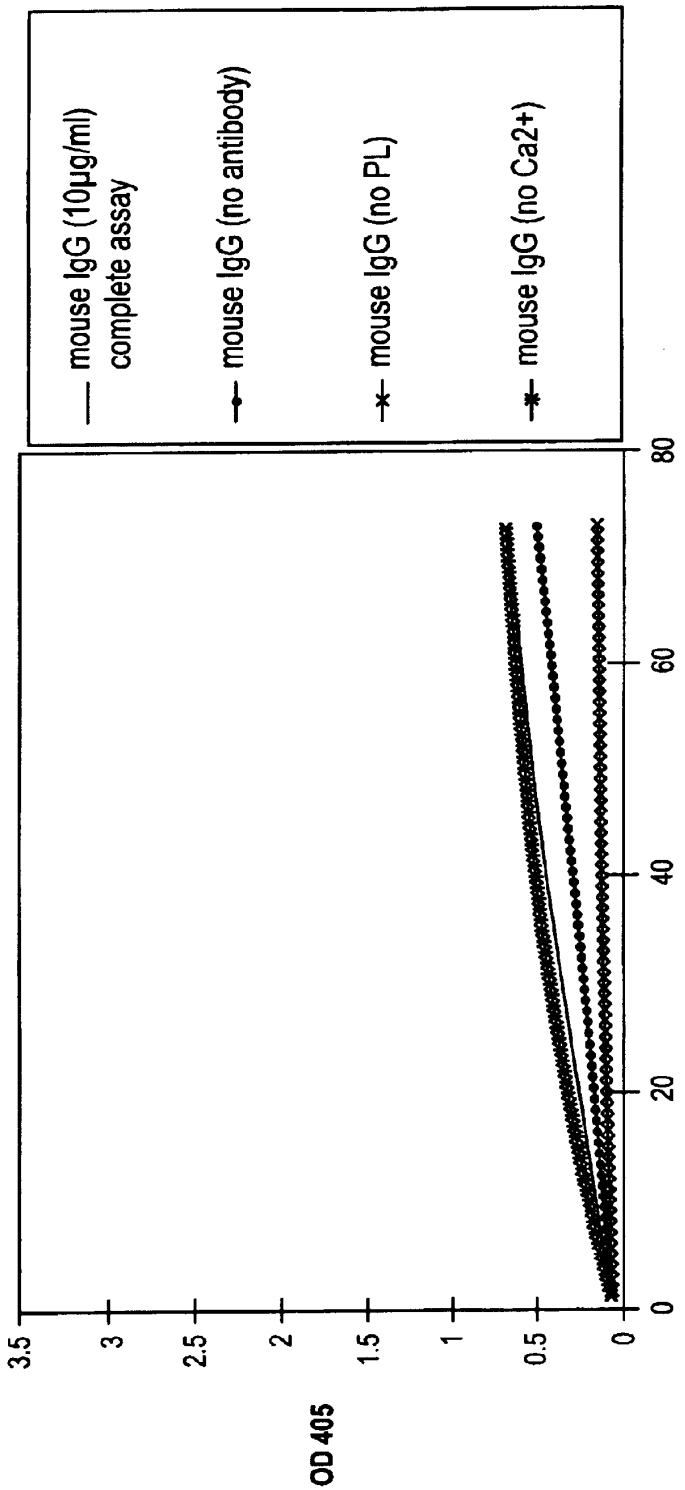

mouse IgG (10µg/ml) complete assay
mouse IgG (no antibody)
mouse IgG (no PL)
mouse IgG (no Ca2+)
OD 405
Scan number
0
0.5
1
1.5
2
2.5
3
3.5
0
20
40
60
80

FIG. 8C

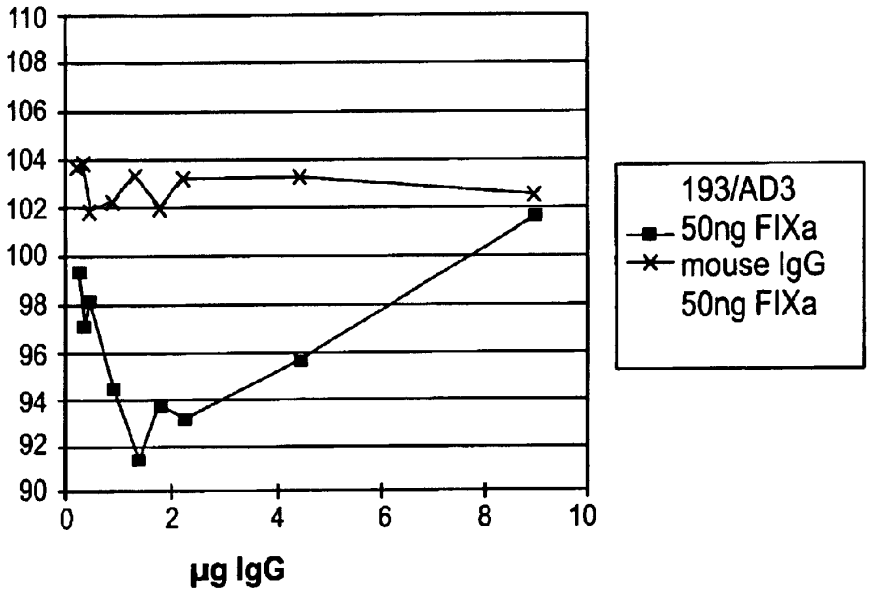

110
108
106
104
102
100
98
96
94
92
90
0
2
4
6
8
10
µg IgG
193/AD3
50ng FIXa
mouse IgG
50ng FIXa

FIG. 9

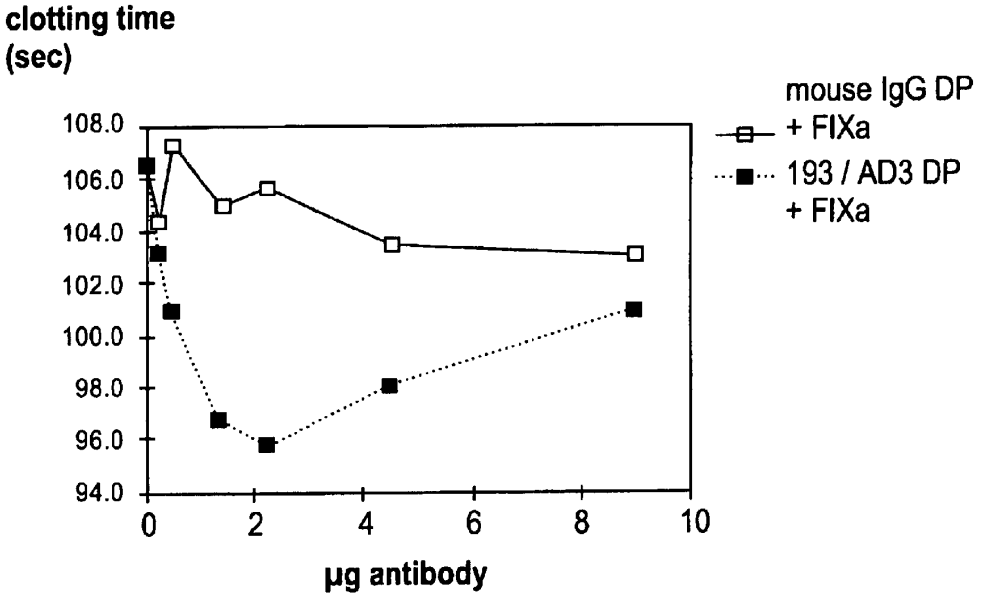

clotting time
(sec)
108.0
106.0
104.0
102.0
100.0
98.0
96.0
94.0
0
2
4
6
8
10
µg antibody
mouse IgG DP
+ FIXa
193 / AD3 DP
+ FIXa

FIG. 10A

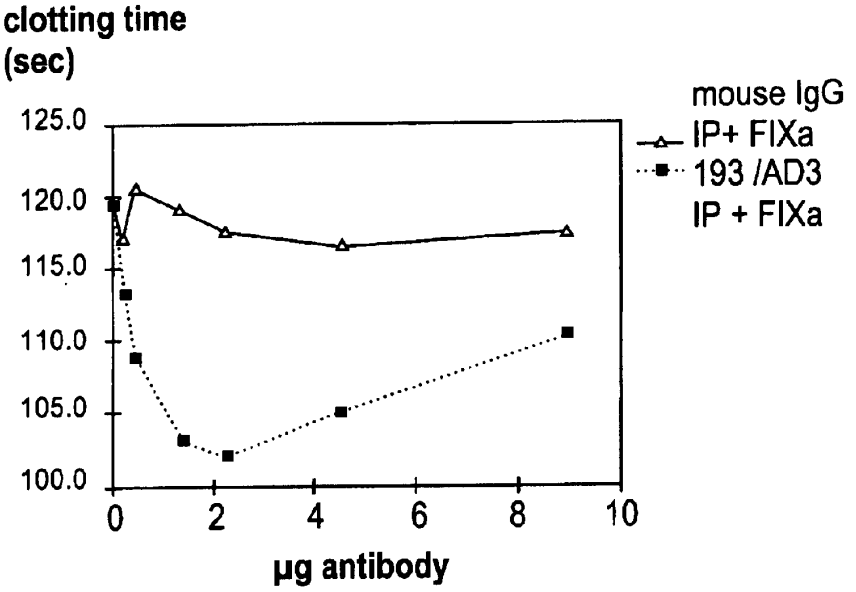

clotting time
(sec)
125.0
120.0
115.0
110.0
105.0
100.0
0
2
4
6
8
10
µg antibody
mouse IgG
IP+ FIXa
193 /AD3
IP + FIXa

FIG. 10B

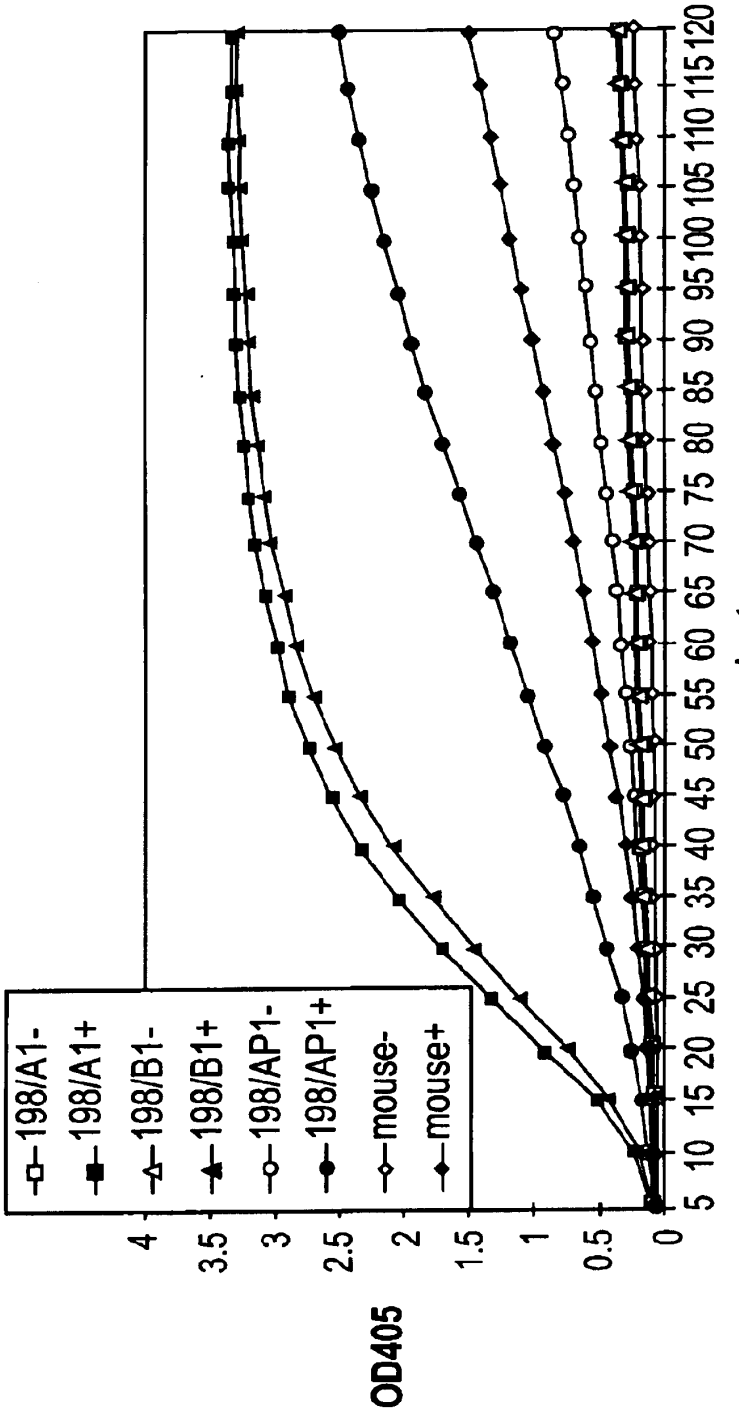

198/A1-
198/A1+
198/B1-
198/B1+
198/AP1-
198/AP1+
mouse-
mouse+
OD405
0
0.5
1
1.5
2
2.5
3
3.5
4
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120
minutes

FIG. 11

Mouse V_H back primers (containing SfiI-site):

VH1BACK-SfiI 5' C ATG CCA TGA CTC GCG GCC CAG CCG GCC ATG GCC SAG GTS MAR CTG CAG
SAG TCW GG 3' (SEQ.ID.NO. 50)

VH1BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTT CAG GAG TCA
GG 3' (SEQ.ID.NO. 51)

VH2BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAT GTG CAG CTT CAG GAG TCR
GG 3' (SEQ.ID.NO. 52)

VH3BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG AAG SAG TCA
GG 3' (SEQ.ID.NO. 53)

VH4/6BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTY CAG CTG CAR CAR TCT
GG 3' (SEQ.ID.NO. 54)

VH5/9BACKsfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTY CAR CTG CAG CAG YCT
GG 3' (SEQ.ID.NO. 55)

VH7BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAR GTG AAG CTG GTG GAR TCT
GG 3' (SEQ.ID.NO. 56)

VH8BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTT CAG CTT CAG CAG TCT
GG 3' (SEQ.ID.NO. 57)

VH10BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAA GTG CAG CTG KTG GAG WCT
GG 3' (SEQ.ID.NO. 58)

VH11BACKSfi 5' GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG ATC CAG TTG CTG CAG TCT
GG 3' (SEQ.ID.NO. 59)

FIG. 12-1

Mouse J_H forward primers (containing 1/2 linker-sequence and AscI-site):

VH1FOR2LiAsc	5' ACC GCC AGA GGC GCG CCC ACC TGA ACC GCC TCC ACC TGA GGA GAC GGT
	GAC CGT GGT CCC TTG GCC CC 3'(SEQ.ID.NO. 60)
JH1FORLiAsc	5' ACC GCC AGA GGC GCG CCC ACC TGA ACC GCC TCC ACC TGA GGA GAC GGT
	GAC CGT GGT CCC 3'(SEQ.ID.NO. 61)
JH2FORLiAsc	5' ACC GCC AGA GGC GCG CCC ACC TGA ACC GCC TCC ACC TGA GGA GAC TGT
	GAG AGT GGT GCC 3'(SEQ.ID.NO. 62)
JH3FORLiAsc	5' ACC GCC AGA GGC GCG CCC ACC TGA ACC GCC TCC ACC TGC AGA GAC AGT
	GAC CAG AGT CCC 3'(SEQ.ID.NO. 63)
JH4FORLiAsc	5' ACC GCC AGA GGC GCG CCC ACC TGA ACC GCC TCC ACC TGA GGA GAC GGT
	GAC TGA GGT TCC 3'(SEQ.ID.NO. 64)

IUPAC-Code: M=A/C, W=A/T, R=A/G, Y=C/T, S=C/G, K=G/T, H=A/C/T, D=A/G/T, V=A/C/G, B=T/C/G.

FIG. 12-2

Primers for cloning mouse V_K genes

Mouse V_K back primers (containing AscI-site and 1/2 linker-sequence):

VK2BACK-LiAscI	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG GAC ATT GAG
	CTC ACC CAG TCT CCA 3' (SEQ.ID.NO. 65)
VK1BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG GAC ATT GTG
	ATG WCA CAG TCT CC 3' (SEQ.ID.NO. 66)
VK2BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG GAT GTT KTG
	ATG ACC CAA ACT CC 3' (SEQ.ID.NO. 67)
VK3BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG GAT ATT GTG
	ATR ACB CAG GCW GC 3' (SEQ.ID.NO. 68)
VK4BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG GAC ATT GTG
	CTG ACM CAR TCT GC 3' (SEQ.ID.NO. 69)
VK5BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG SAA AWT GTK
	CTC ACC CAG TCT CC 3' (SEQ.ID.NO. 70)
VK6BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG GAY ATY VWG
	ATG ACM CAG WCT CC 3' (SEQ.ID.NO. 71)
VK7BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG CAA ATT GTT
	CTC ACC CAG TCT CC 3' (SEQ.ID.NO. 72)
VK8BACKLi Asc	5' GGT TCA GAT GGG CGC GCC TCT GGC GGT GGC GGA TCG TCA TTA TTG
	CAG GTG CTT GTG GG 3' (SEQ.ID.NO. 73)

FIG. 13-1

Mouse J_K forward primers (containing NotI-site):

JK1NOT10	5' GAG TCA TTC TGC GGC CGC CCG TTT GAT TTC CAG CTT GGT GCC 3' (SEQ.ID.NO. 74)
JK2NOT10	5' GAG TCA TTC TGC GGC CGC CCG TTT TAT TTC CAG CTT GGT CCC 3' (SEQ.ID.NO. 75)
JK3NOT10	5' GAG TCA TTC TGC GGC CGC CCG TTT TAT TTC CAG TCT GGT CCC 3' (SEQ.ID.NO. 76)
JK4NOT10	5' GAG TCA TTC TGC GGC CGC CCG TTT TAT TTC CAA CTT TGT CCC 3' (SEQ.ID.NO. 77)
JK5NOT10	5' GAG TCA TTC TGC GGC CGC CCG TTT CAG CTC CAG CTT GGT CCC 3' (SEQ.ID.NO. 78)

IUPAC-Code: K=G/T, M=A/C, W=A/T, R=A/G, Y=C/T, S=C/G, H=A/C/T, D=A/G/T, V=A/C/G, B=T/C/G.

FIG. 13-2

VH

+1 E V K L V E S G P E L K K P G
1 GAG GTG AAG CTG GTG GAG TCT GGA CCT GAG CTG AAG AAG CCT GGA

+1 E T V K I S C K A S G Y I F T
46 GAG ACA GTC AAG ATC TCC TGC AAG GCT TCT GGG TAT ATC TTC ACA

+1 N Y G M N W V K Q A P G K G L
91 AAC TAT GGA ATG AAC TGG GTG AAG CAG GCT CCA GGA AAG GGT TTA

+1 K W M G W I N T Y T G E P T Y
136 AAG TGG ATG GGC TGG ATA AAC ACC TAC ACT GGA GAG CCA ACA TAT

+1 A D D F K G R F A F S L E T S
181 GCT GAT GAC TTC AAG GGA CGG TTT GCC TTC TCT TTG GAA ACC TCT

+1 A S T A Y L Q I N N L K N E D
226 GCC AGC ACT GCC TAT TTG CAG ATC AAC AAC CTC AAA AAT GAG GAC

+1 T A T Y F C A L **Y G N S P K G**
271 ACG GCT ACA TAT TTC TGT GCA TTA **TAT GGT AAC TCC CCT AAG GGG**

linker

+1 **F A Y** W G Q G T L V T V S A G
316 **TTT GCT TAC** TGG GGC CAA GGG ACT CTG GTC ACT GTC TCT GCA GGT

VL

+1 G G G S G G R A S G G G G S D
361 GGA GGC GGT TCA GGT GGG CGC GCC TCT GGC GGT GGC GGA TCG GAT

+1 I Q M T Q S P K F L L V S A G
406 ATT CAG ATG ACA CAG TCT CCC AAA TTC CTG CTT GTA TCA GCA GGA

FIG. 14-1

```
+1    D   R   V   T   I   T   C   K   A   S   Q   S   V   S   N
451   GAC AGG GTT ACC ATA ACC TGC AAG GCC AGT CAG AGT GTG AGT AAT

+1    D   V   A   W   Y   Q   Q   K   P   G   Q   S   P   K   L
496   GAT GTA GCT TGG TAC CAA CAG AAG CCG GGG CAG TCT CCT AAA CTA

+1    L   M   Y   Y   A   S   N   R   Y   T   G   V   P   D   R
541   CTG ATG TAC TAT GCA TCC AAT CGC TAC ACT GGA GTC CCT GAT CGC

+1    F   T   G   S   G   Y   G   T   D   F   T   F   T   I   S
586   TTC ACT GGC AGT GGA TAT GGG ACG GAT TTC ACT TTC ACC ATC AGC

+1    T   V   Q   A   E   D   L   A   V   Y   F   C   Q   Q   D
631   ACT GTG CAG GCT GAA GAC CTG GCA GTT TAT TTC TGT CAG CAG GAT

+1    Y   G   S   P   P   T   F   G   G   G   T   K   L   E   I
676   TAT GGC TCT CCT CCC ACG TTC GGA GGG GGC ACC AAG CTG GAA ATT

+1    K   R
721   AAA CGG
```

FIG. 14-2

VH

```
+1    E   V   Q   L   V   E   S   G   G   G   L   V   K   P   G
1   GAA GTG CAG CTG GTG GAG TCT GGG GGA GGC CTA GTG AAG CCT GGA

+1    G   S   L   K   L   S   C   A   A   S   G   F   T   F   S
46  GGG TCC CTG AAA CTC TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT

+1    T   Y   T   M   S   W   V   R   Q   T   P   E   K   R   L
91  ACC TAT ACC ATG TCT TGG GTT CGC CAG ACT CCG GAG AAG AGG CTG

+1    E   W   V   A   T   I   S   S   G   G   S   Y   T   Y   Y
136 GAG TGG GTC GCA ACC ATT AGT AGT GGT GGT AGT TAC ACC TAC TAT

+1    P   D   S   V   R   G   R   F   T   I   S   R   D   N   A
181 CCA GAC AGT GTG AGG GGC CGA TTC ACC ATC TCC AGA GAC AAT GCC

+1    K   N   T   L   Y   L   Q   M   S   S   L   K   S   E   D
226 AAG AAC ACC CTG TAC CTG CAA ATG AGC AGT CTG AAG TCT GAG GAC

+1    T   A   M   Y   Y   C   T   R   D   G   G   H   G   Y   G
271 ACA GCC ATG TAT TAC TGT ACA AGA GAT GGG GGA CAC GGG TAC GGT

+1    S   S   F   D   Y   W   G   Q   G   T   T   L   T   V   S
316 AGT AGC TTT GAC TAC TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC
```

linker

```
+1    S   G   G   G   G   S   G   G   R   A   S   G   G   G   G
361 TCA GGT GGA GGC GGT TCA GGT GGG CGC GCC TCT GGC GGT GGC GGA
```

VL

```
+1    S   Q   I   V   L   T   Q   S   P   L   S   L   P   V   S
406 TCG CAA ATT GTG CTC ACC CAG TCT CCA CTC TCC CTG CCT GTC AGT
```

FIG. 15-1

```
+1    L   G   D   Q   A   S   I   S   C   R   S   S   Q   S   I
451   CTT GGA GAT CAA GCC TCC ATC TCT TGC AGA TCT AGT CAG AGC ATT

+1    V   H   S   N   G   N   T   Y   L   E   W   Y   L   Q   K
496   GTA CAT AGT AAT GGA AAC ACC TAT TTA GAA TGG TAC CTG CAG AAA

+1    P   G   Q   S   P   K   L   L   I   Y   K   V   S   N   R
541   CCA GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT TCC AAC CGA

+1    F   S   G   V   P   D   K   F   S   G   S   G   S   G   T
586   TTT TCT GGG GTC CCA GAC AAA TTC AGT GGC AGT GGA TCA GGG ACA

+1    D   F   T   L   K   I   S   R   V   E   A   E   D   L   G
631   GAT TTC ACA CTC AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG GGA

+1    V   Y   Y   C   F   Q   G   S   H   V   P   W   T   F   G
676   GTT TAT TAC TGC TTT CAA GGT TCA CAT GTT CCG TGG ACG TTC GGT

+1    G   G   T   K   L   E   I   K   R
721   GGA GGC ACC AAG CTG GAA ATC AAA CGG
```

FIG. 15-2

```
+1  E   V   Q   L   Q   E   S   G   G   G   L   V   K   P   G
1   GAG GTG CAG CTT CAG GAG TCA GGG GGA GGC TTA GTG AAG CCT GGA

+1   G   S   L   K   L   S   C   A   A   S   G   F   T   F   S
46   GGG TCC CTG AAA CTC TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT

+1    S   Y   T   M   S   W   V   R   Q   T   P   E   K   R   L
91   AGC TAT ACC ATG TCT TGG GTT CGC CAG ACT CCG GAG AAG AGG CTG

+1    E   W   V   A   T   I   S   S   G   G   S   S   T   Y   Y
136  GAG TGG GTC GCA ACC ATT AGT AGT GGT GGT AGT TCC ACC TAC TAT

+1    P   D   S   V   K   G   R   F   T   I   S   R   D   N   A
181  CCA GAC AGT GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT GCC

+1    K   N   T   L   Y   L   Q   M   S   S   L   R   S   E   D
226  AAG AAC ACC CTG TAC CTG CAA ATG AGC AGT CTG AGG TCT GAG GAC

+1    T   A   M   Y   Y   C   T   R   E   G   G   G   F   T   V
271  ACA GCC ATG TAT TAC TGT ACA AGA GAG GGG GGT GGT TTC ACC GTC

+1    N   W   Y   F   D   V   W   G   A   G   T   L   V   T   V
316  AAC TGG TAC TTC GAT GTC TGG GGC GCA GGG ACT CTG GTC ACT GTC

                linker
+1    S   A   G   G   G   G   S   G   G   R   A   S   G   G   G
361  TCT GCA GGT GGA GGC GGT TCA GGT GGG CGC GCC TCT GGC GGT GGC

              VL
+1    G   S   E   N   V   L   T   Q   S   P   A   S   L   A   V
406  GGA TCG GAA AAT GTG CTC ACC CAG TCT CCA GCT TCT TTG GCT GTG
```

FIG. 16-1

```
+1  S   L   G   Q   R   A   T   I   S   C   R   A   S   E   S
451 TCT CTA GGG CAG AGG GCC ACC ATA TCC TGC AGA GCC AGT GAA AGT

+1    V   D   S   Y   G   Y   N   F   M   H   W   Y   Q   Q   I
496 GTT GAT AGT TAT GGC TAT AAT TTT ATG CAC TGG TAT CAG CAG ATA

+1    P   G   Q   P   P   K   L   L   I   Y   R   A   S   N   L
541 CCA GGA CAG CCA CCC AAA CTC CTC ATC TAT CGT GCA TCC AAC CTA

+1    E   S   G   I   P   A   R   F   S   G   S   G   S   R   T
586 GAG TCT GGG ATC CCT GCC AGG TTC AGT GGC AGT GGG TCT AGG ACA

+1    D   F   T   L   T   I   N   P   V   E   A   D   D   V   A
631 GAC TTC ACC CTC ACC ATT AAT CCT GTG GAG GCT GAT GAT GTT GCA

+1    T   Y   Y   C   Q   Q   S   N   E   D   P   L   T   F   G
676 ACC TAT TAC TGT CAG CAA AGT AAT GAG GAT CCG CTC ACG TTC GGT

+1    T   G   T   R   L   E   I   K   R
721 ACT GGG ACC AGA CTG GAA ATA AAA CGG
```

FIG. 16-2

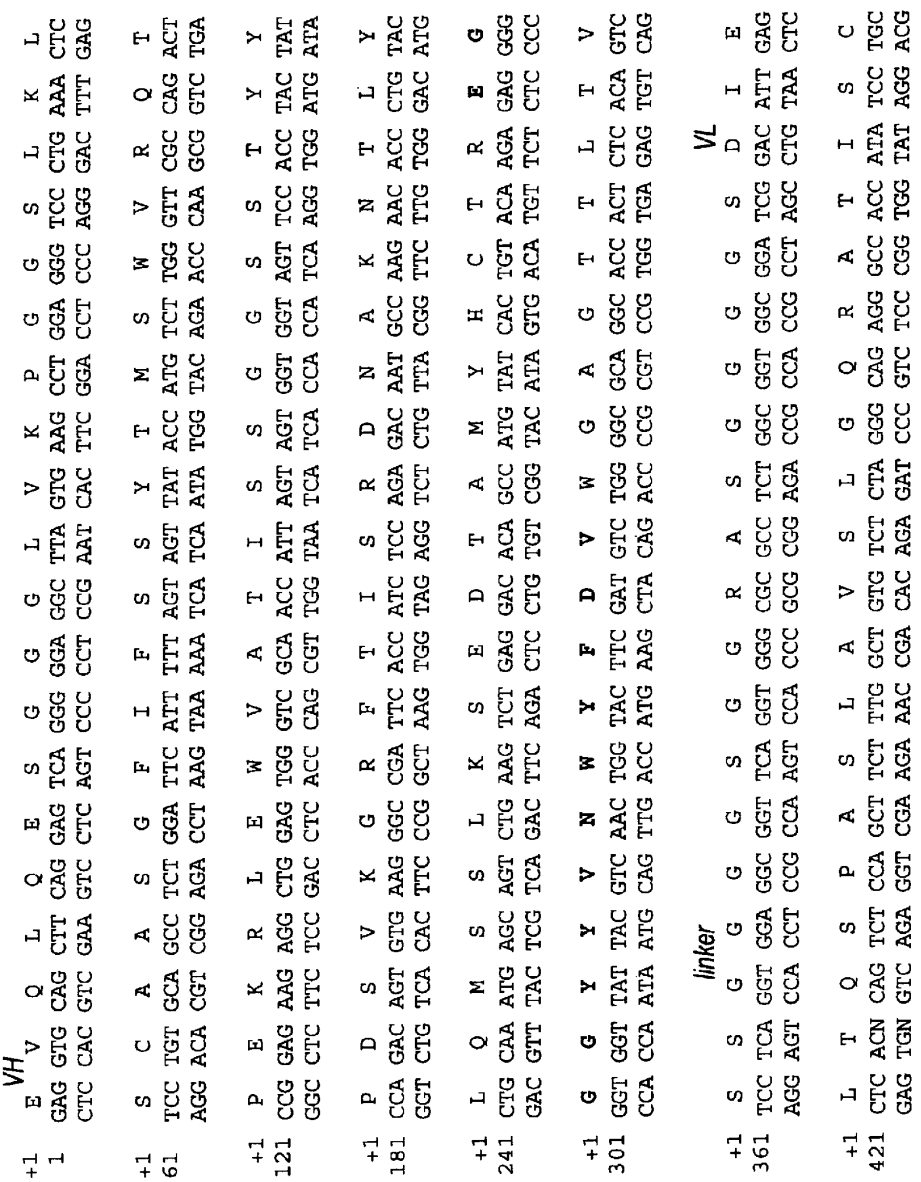


FIG. 17-1

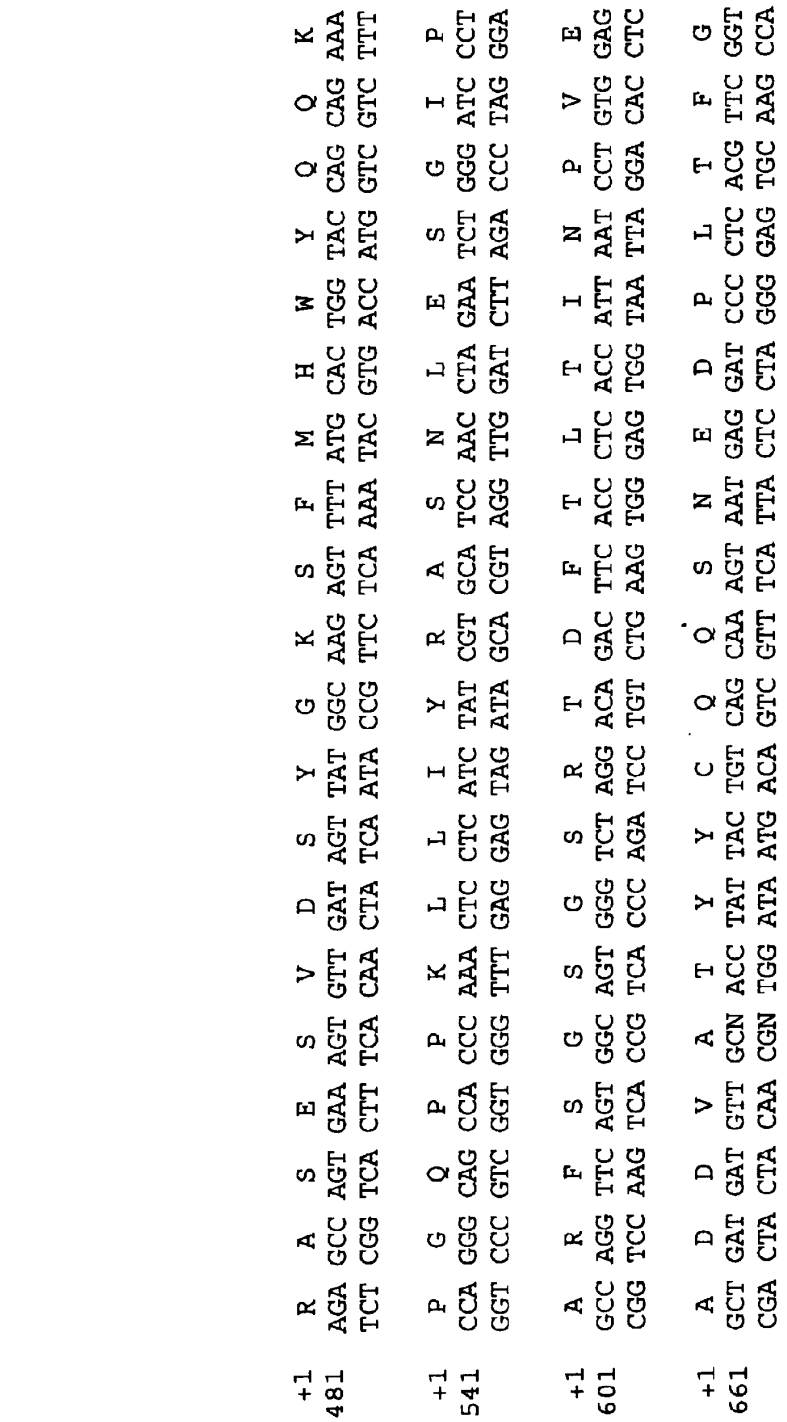

+1 R A S E S V D S Y G K S F M H W Y Q Q K
481 AGA GCC AGT GAA AGT GTT GAT AGT TAT GGC AAG AGT TTT ATG CAC TGG TAC CAG CAG AAA
TCT CGG TCA CTT TCA CAA CTA TCA ATA CCG TTC TCA AAA TAC GTG ACC ATG GTC GTC TTT
+1 P G Q P P K L L I Y R A S N L E S G I P
541 CCA GGG CAG CCA CCC AAA CTC CTC ATC TAT CGT GCA TCC AAC CTA GAA TCT GGG ATC CCT
GGT CCC GTC GGT GGG TTT GAG GAG TAG ATA GCA CGT AGG TTG GAT CTT AGA CCC TAG GGA
+1 A R F S G S G S R T D F T L T I N P V E
601 GCC AGG TTC AGT GGC AGT GGG TCT AGG ACA GAC TTC ACC CTC ACC ATT AAT CCT GTG GAG
CGG TCC AAG TCA CCG TCA CCC AGA TCC TGT CTG AAG TGG GAG TGG TAA TTA GGA CAC CTC
+1 A D D V A T Y Y C Q Q S N E D P L T F G
661 GCT GAT GAT GTT GCN ACC TAT TAC TGT CAG CAA AGT AAT GAG GAT CCC CTC ACG TTC GGT
CGA CTA CTA CAA CGN TGG ATA ATG ACA GTC GTT TCA TTA CTC CTA GGG GAG TGC AAG CCA
+1 A G T R L E I K R
721 GCT GGG ACC AGA CTG GAA ATA AAA CGG
CGA CCC TGG TCT GAC CTT TAT TTT GCC

FIG. 17-2

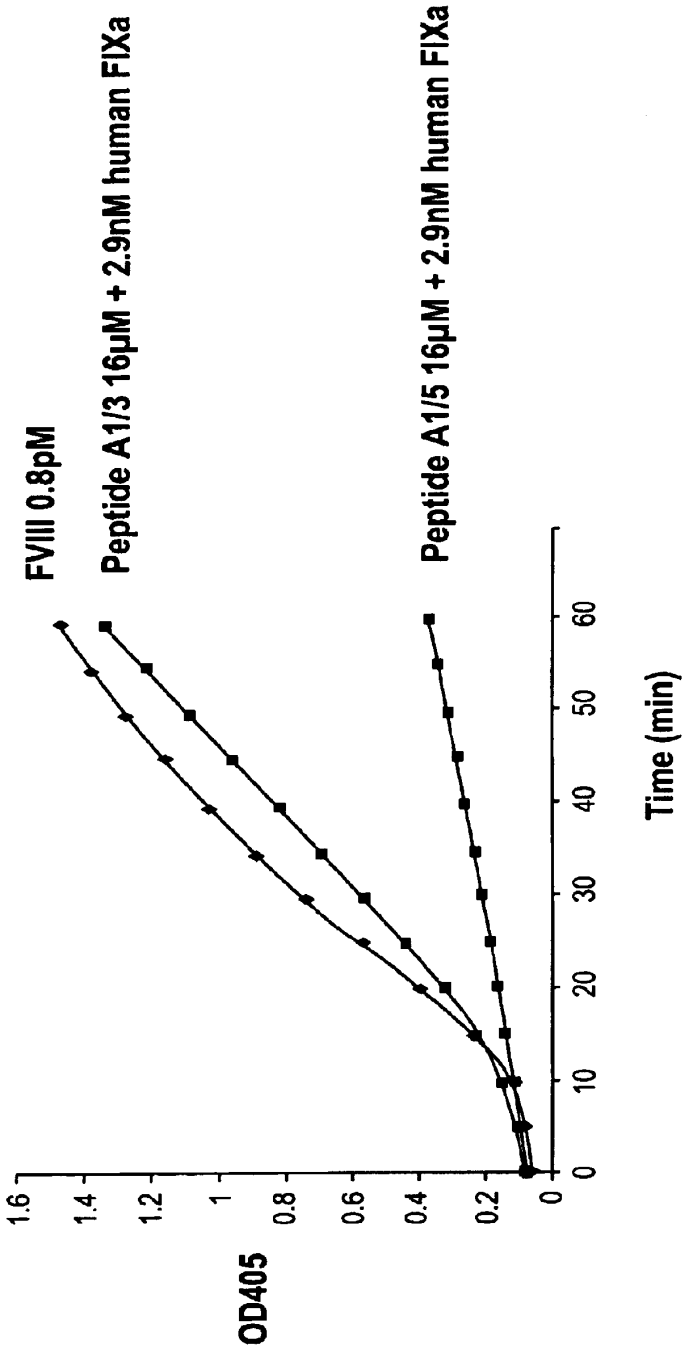

FVIII 0.8pM
Peptide A1/3 16µM + 2.9nM human FIXa
Peptide A1/5 16µM + 2.9nM human FIXa
OD405
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
Time (min)

FIG. 18

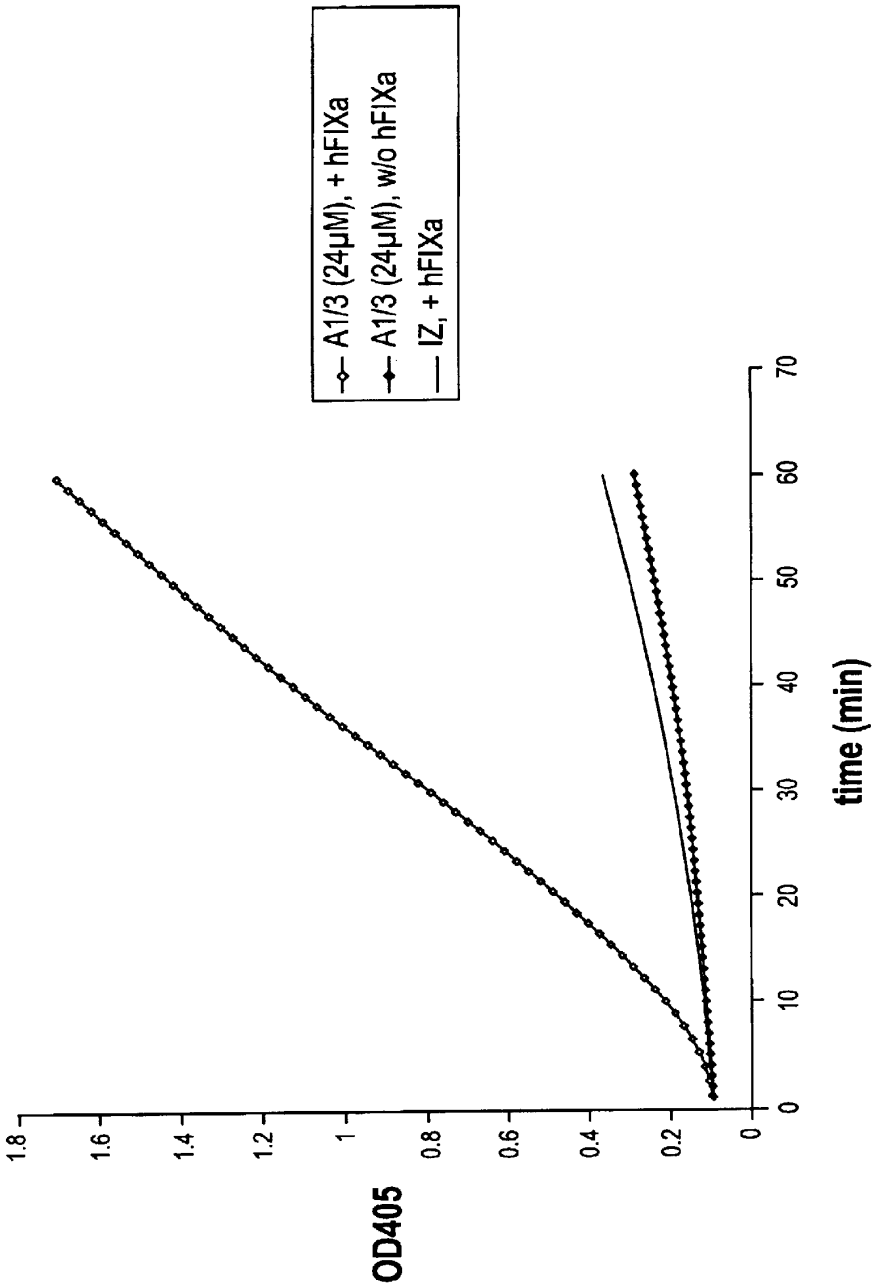

A1/3 (24μM), + hFIXa
A1/3 (24μM), w/o hFIXa
IZ, + hFIXa
OD405
time (min)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
0
10
20
30
40
50
60
70

FIG. 19

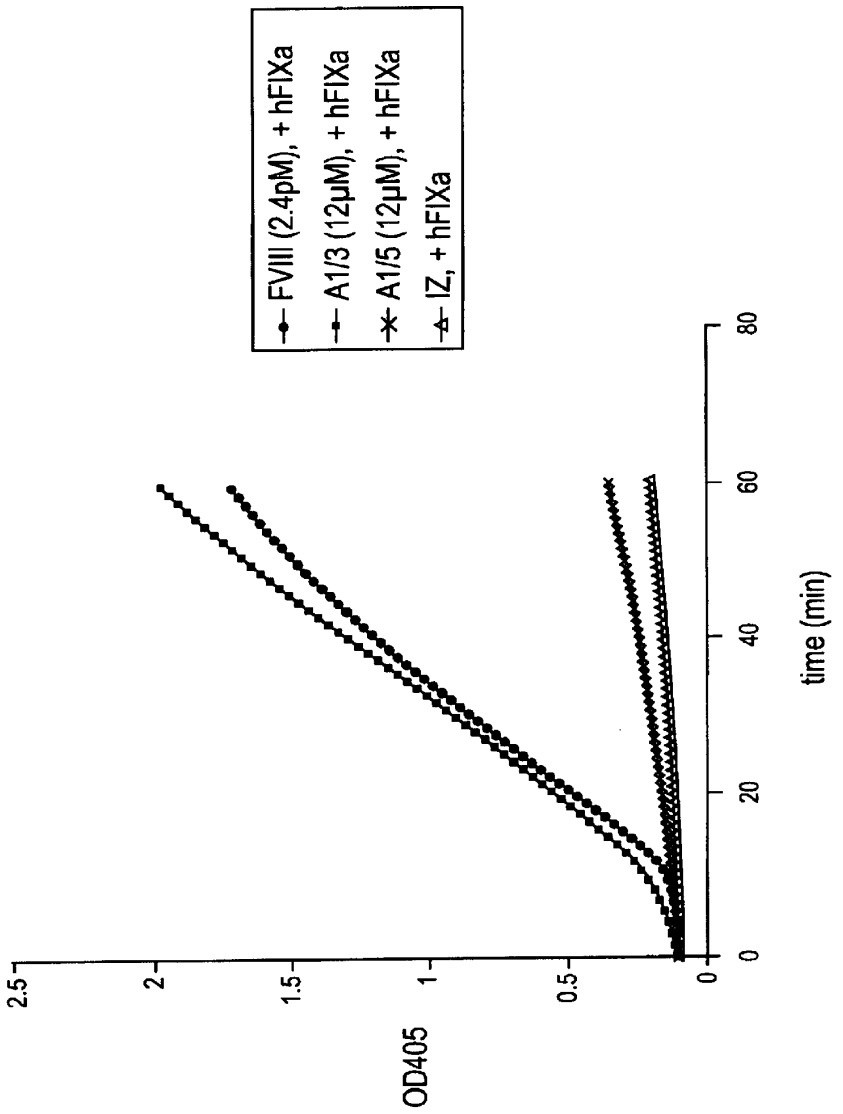

FVIII (2.4pM), + hFIXa
A1/3 (12μM), + hFIXa
A1/5 (12μM), + hFIXa
IZ, + hFIXa
OD405
2.5
2
1.5
1
0.5
0
0
20
40
60
80
time (min)

FIG. 20

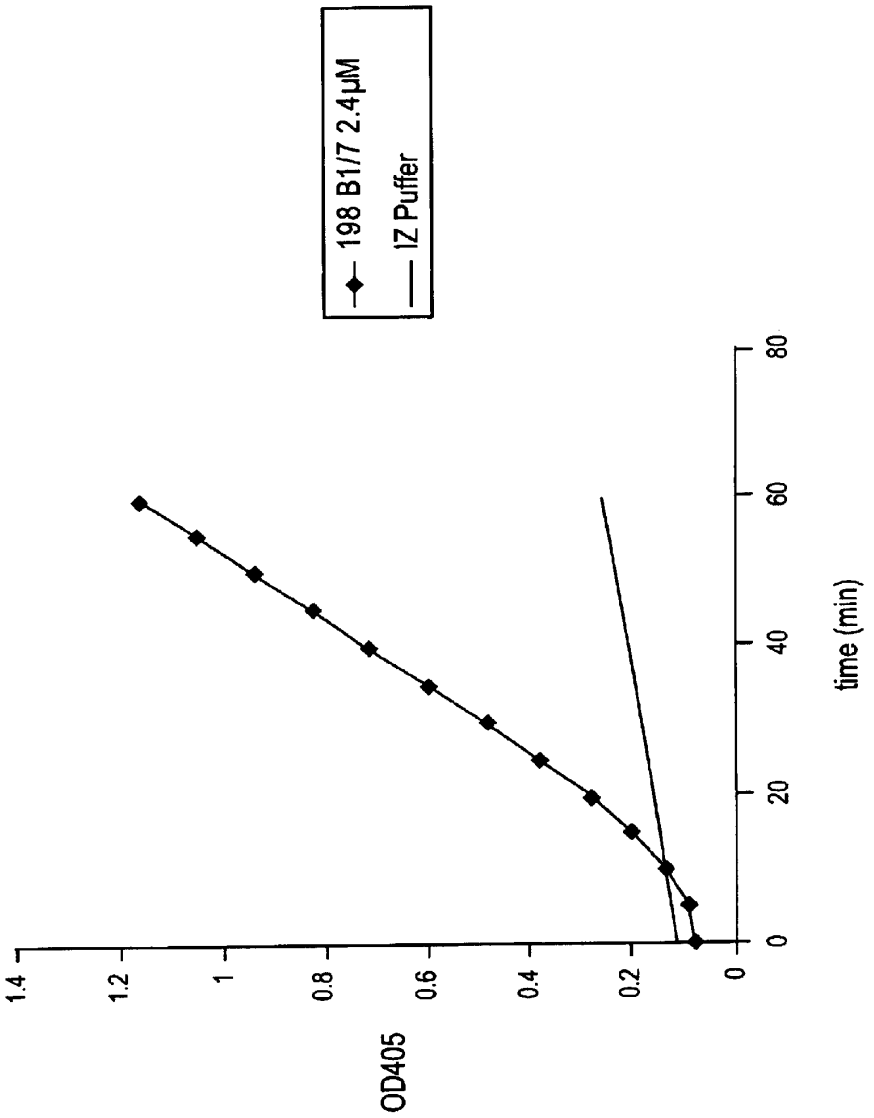

198 B1/7 2.4µM
IZ Puffer
OD405
time (min)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
20
40
60
80

FIG. 21

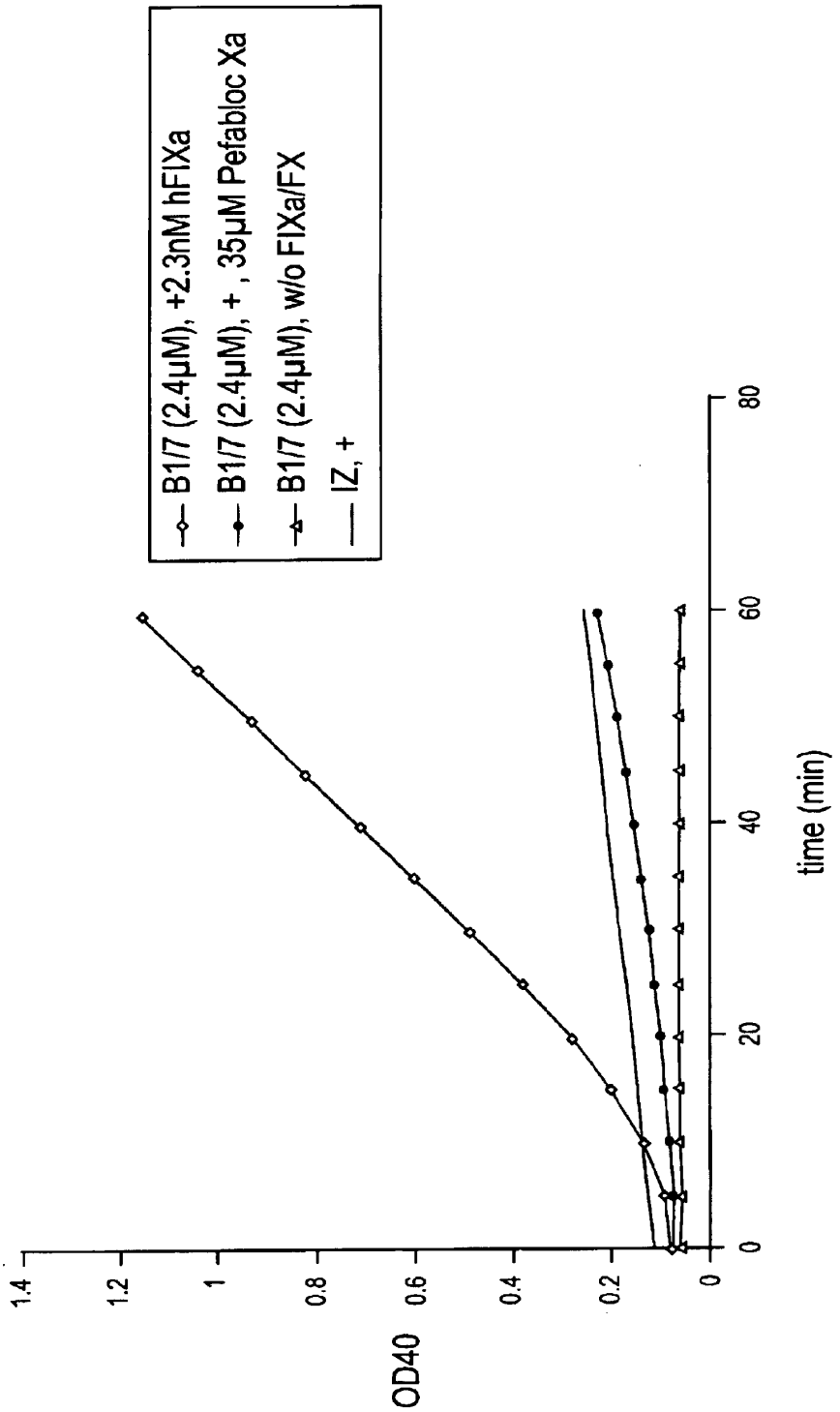

B1/7 (2.4µM), +2.3nM hFIXa
B1/7 (2.4µM), + , 35µM Pefabloc Xa
B1/7 (2.4µM), w/o FIXa/FX
IZ, +
OD40
time (min)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
20
40
60
80

FIG. 22

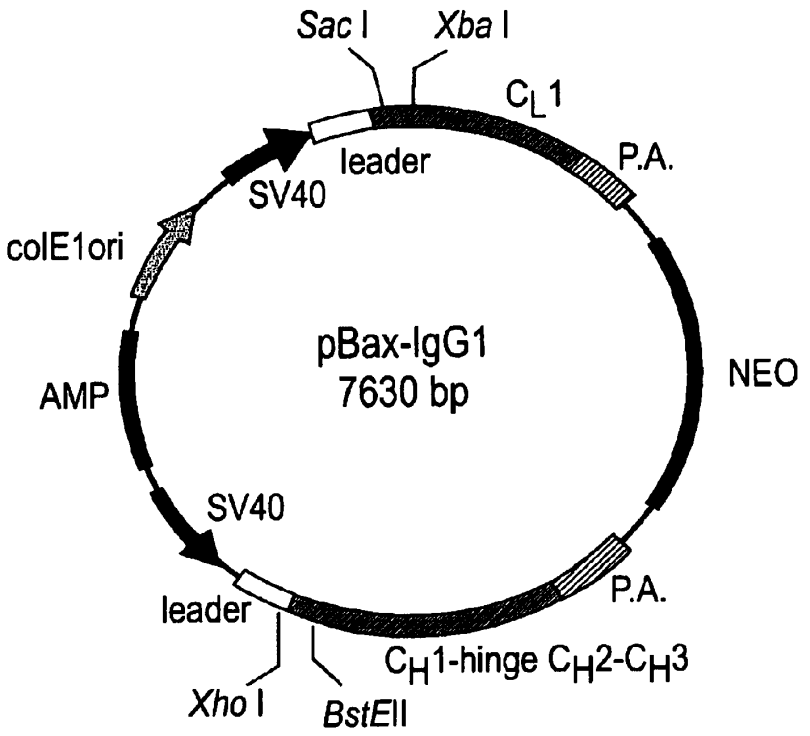

Sac I
Xba I
C_L1
leader
P.A.
SV40
colE1ori
pBax-IgG1
7630 bp
NEO
AMP
SV40
P.A.
leader
C_H1-hinge C_H2-C_H3
Xho I
BstEII

FIG. 23

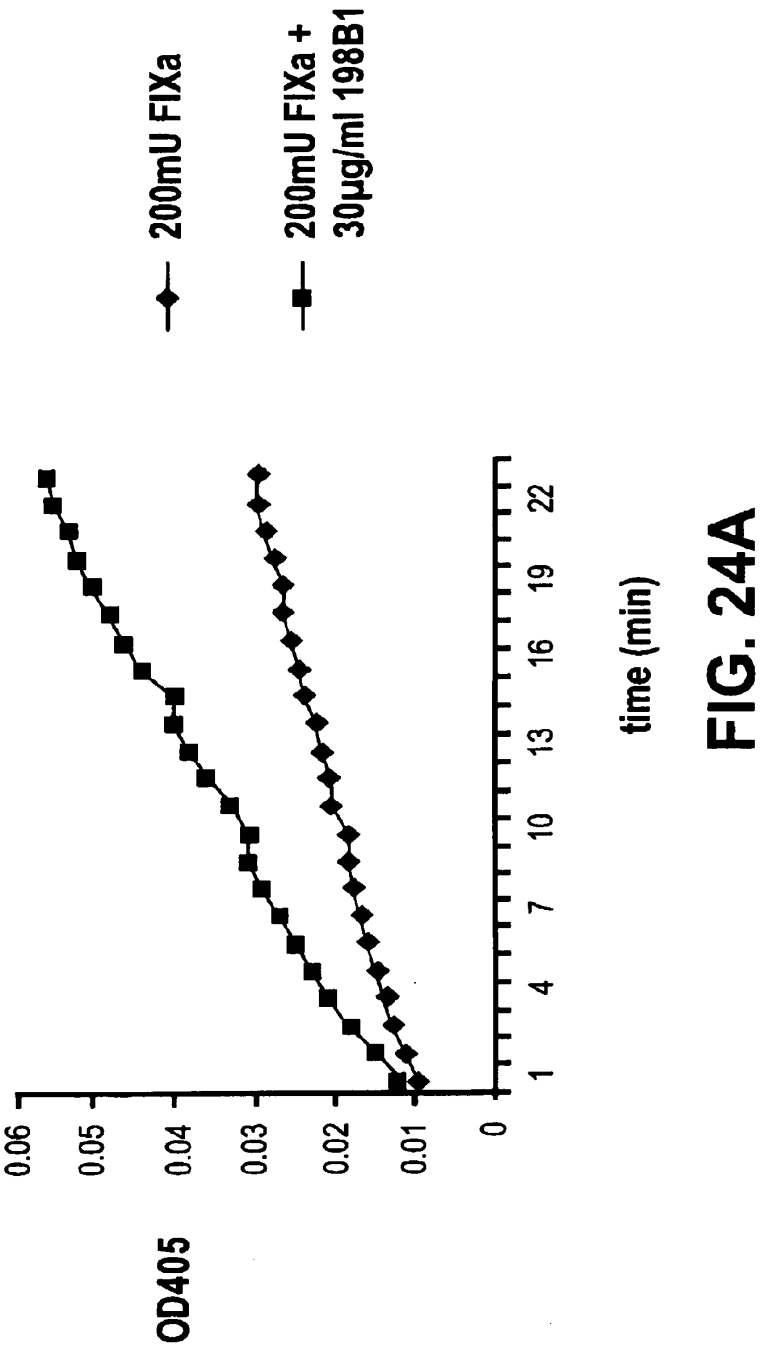

200mU FIXa
200mU FIXa + 30µg/ml 198B1
OD405
0.06
0.05
0.04
0.03
0.02
0.01
0
1
4
7
10
13
16
19
22
time (min)

FIG. 24A

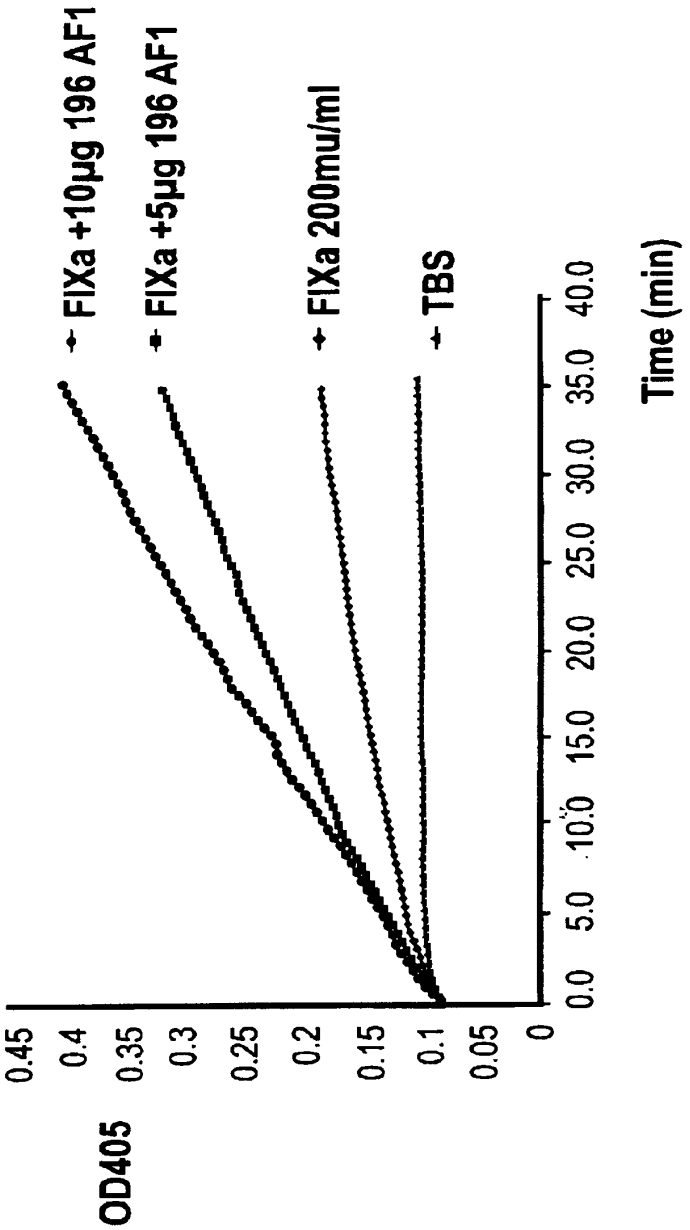

FIXa +10µg 196 AF1
FIXa +5µg 196 AF1
FIXa 200mu/ml
TBS
OD405
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
Time (min)

FIG. 24B

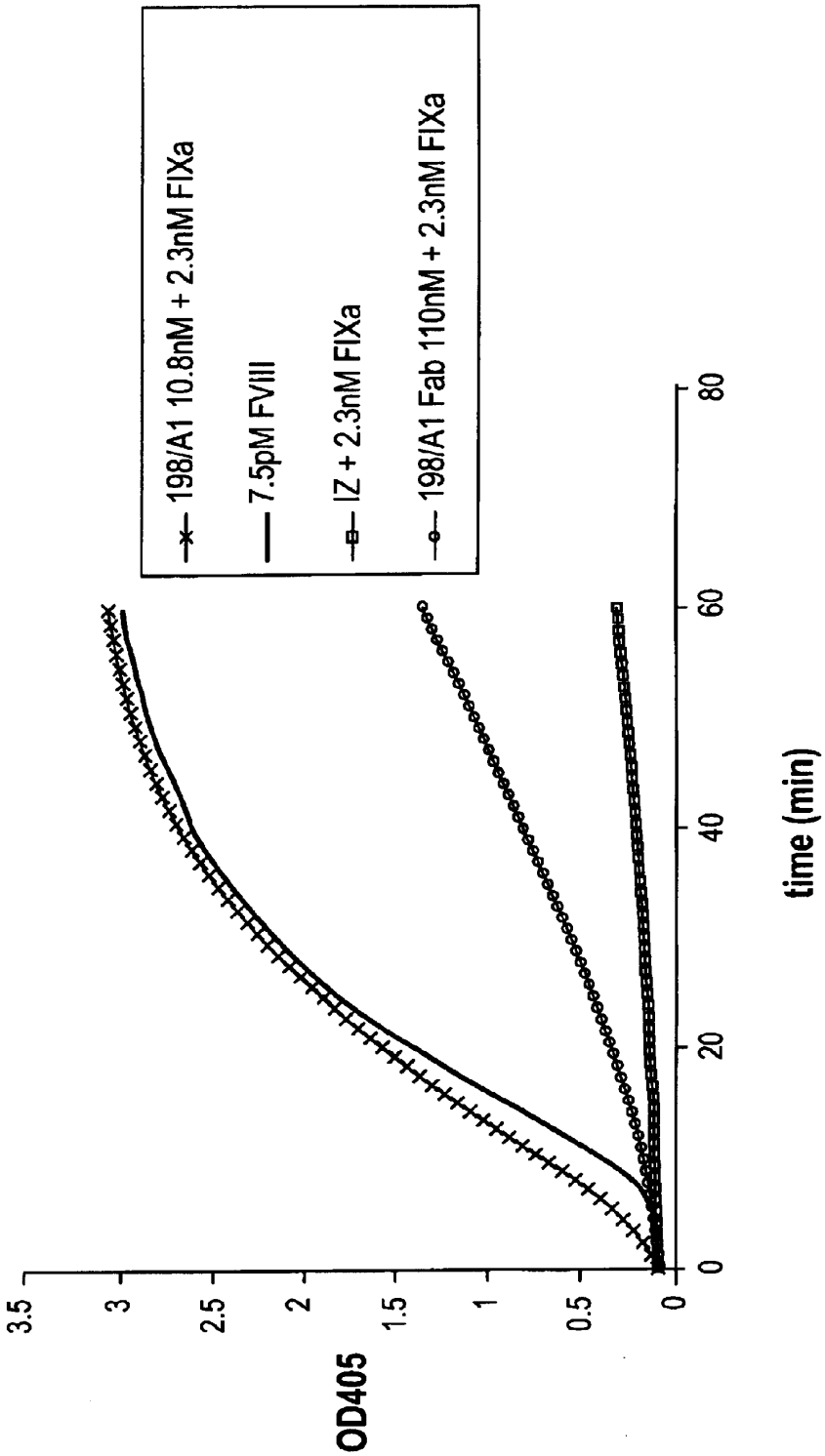

198/A1 10.8nM + 2.3nM FIXa
7.5pM FVIII
IZ + 2.3nM FIXa
198/A1 Fab 110nM + 2.3nM FIXa
OD405
3.5
3
2.5
2
1.5
1
0.5
0
0
20
40
60
80
time (min)

FIG. 25

PelB-Leader

```
+1   M   K   Y   L   L   P   T   A   A   A   G   L   L   L
1   ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA
    TAC TTT ATG GAT AAC GGA TGC CGT CGG CGA CCT AAC AAT AAT

                                                VH
+1   L   A   A   Q   P   A   M   A | E   V   K   L   V   E
43  CTC GCG GCC CAG CCG GCC ATG GCG|GAG GTG AAG CTG GTG GAG
    GAG CGC CGG GTC GGC CGG TAC CGC|CTC CAC TTC GAC CAC CTC

+1   S   G   G   G   L   V   K   P   G   G   S   L   K   L
85  TCT GGG GGA GGC TTA GTG AAG CCT GGA GGG TCC CTG AAA CTC
    AGA CCC CCT CCG AAT CAC TTC GGA CCT CCC AGG GAC TTT GAG

+1   S   C   A   A   S   G   F   T   F   S   S   Y   T   M
127 TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT AGC TAT ACC ATG
    AGG ACA CGT CGG AGA CCT AAG TGA AAG TCA TCG ATA TGG TAC

+1   S   W   V   R   Q   T   P   E   K   R   L   E   W   V
169 TCT TGG GTT CGC CAG ACT CCG GAG AAG AGG CTG GAG TGG GTC
    AGA ACC CAA GCG GTC TGA GGC CTC TTC TCC GAC CTC ACC CAG

+1   A   T   I   S   S   G   G   S   S   T   Y   Y   P   D
211 GCA ACC ATT AGT AGT GGN GGT AGT TCC ACC TAC TAT CCA GAC
    CGT TGG TAA TCA TCA CCN CCA TCA AGG TGG ATG ATA GGT CTG

+1   S   V   K   G   R   F   T   I   S   R   D   N   A   K
253 AGT GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT GCC AAG
    TCA CAC TTC CCG GCT AAG TGG TAG AGG TCT CTG TTA CGG TTC

+1   N   T   L   Y   L   Q   M   S   S   L   R   S   E   D
295 AAC ACC CTG TAC CTG CAA ATG AGC AGT CTG AGG TCT GAG GAC
    TTG TGG GAC ATG GAC GTT TAC TCG TCA GAC TCC AGA CTC CTG

+1   T   A   M   Y   Y   C   T   R   E   G   G   G   F   T
337 ACA GCC ATG TAT TAC TGT ACA AGA GAG GGG GGT GGT TTC ACC
    TGT CGG TAC ATA ATG ACA TGT TCT CTC CCC CCA CCA AAG TGG

+1   V   N   W   Y   F   D   V   W   G   A   G   T   S   V
379 GTC AAC TGG TAC TTC GAT GTC TGG GGC GCA GGA ACC TCA GTC
    CAG TTG ACC ATG AAG CTA CAG ACC CCG CGT CCT TGG AGT CAG

                                    linker
+1   T   V   S   S | G   G   G   G   S   G   G   R   A   S
421 ACC GTC TCC TCA|GGT GGA GGC GGT TCA GGT GGG CGC GCC TCT
    TGG CAG AGG AGT|CCA CCT CCG CCA AGT CCA CCC GCG CGG AGA
```

FIG. 26-1

```
                                        VL
  +1  G   G   G   G   S  |D   I   V   L   T   Q   S   P   A
463  GGC GGT GGC GGA TCG |GAC ATT GTG CTG ACA CAG TCT CCA GCT
     CCG CCA CCG CCT AGC |CTG TAA CAC GAC TGT GTC AGA GGT CGA

  +1  S   L   A   V   S   L   G   Q   R   A   T   I   S   C
505  TCT TTG GCT GTG TCT CTA GGG CAG AGG GCC ACC ATA TCC TGC
     AGA AAC CGA CAC AGA GAT CCC GTC TCC CGG TGG TAT AGG ACG

  +1  R   A   S   E   S   V   D   S   Y   G   Y   N   F   M
547  AGA GCC AGT GAA AGT GTT GAT AGT TAT GGC TAT AAT TTT ATG
     TCT CGG TCA CTT TCA CAA CTA TCA ATA CCG ATA TTA AAA TAC

  +1  H   W   Y   Q   Q   I   P   G   Q   P   P   K   L   L
589  CAC TGG TAT CAG CAG ATA CCA GGA CAG CCA CCC AAA CTC CTC
     GTG ACC ATA GTC GTC TAT GGT CCT GTC GGT GGG TTT GAG GAG

  +1  I   Y   R   A   S   N   L   E   S   G   I   P   A   R
631  ATC TAT CGT GCA TCC AAC CTA GAG TCT GGG ATC CCT GCC AGG
     TAG ATA GCA CGT AGG TTG GAT CTC AGA CCC TAG GGA CGG TCC

  +1  F   S   G   S   G   S   R   T   D   F   T   L   T   I
673  TTC AGT GGC AGT GGG TCT AGG ACA GAC TTC ACC CTC ACC ATT
     AAG TCA CCG TCA CCC AGA TCC TGT CTG AAG TGG GAG TGG TAA

  +1  N   P   V   E   A   D   D   V   A   T   Y   Y   C   Q
715  AAT CCT GTG GAG GCT GAT GAT GTT GCA ACC TAT TAC TGT CAG
     TTA GGA CAC CTC CGA CTA CTA CAA CGT TGG ATA ATG ACA GTC

  +1  Q   S   N   E   D   P   L   T   F   G   T   G   T   R
757  CAA AGT AAT GAG GAT CCG CTC ACG TTC GGT ACT GGG ACC AGA
     GTT TCA TTA CTC CTA GGC GAG TGC AAG CCA TGA CCC TGG TCT

                                Spacer          Alkaline phosphatase
  +1  L   E   I   K   R  |A   A   A   A  |R   A   P   E   M
799  CTG GAA ATA AAA CGG |GCG GCC GCA GCC |CGG GCA CCA GAA ATG
     GAC CTT TAT TTT GCC |CGC CGG CGT CGG |GCC CGT GGT CTT TAC

  +1  P   V   L   E   N   R   A   A   Q   G   D   I   T   A
841  CCT GTT CTG GAA AAC CGG GCT GCT CAG GGC GAT ATT ACT GCA
     GGA CAA GAC CTT TTG GCC CGA CGA GTC CCG CTA TAA TGA CGT

  +1  P   G   G   A   R   R   L   T   G   D   Q   T   A   A
883  CCC GGC GGT GCT CGC CGT TTA ACG GGT GAT CAG ACT GCC GCT
     GGG CCG CCA CGA GCG GCA AAT TGC CCA CTA GTC TGA CGG CGA

  +1  L   R   D   S   L   S   D   K   P   A   K   N   I   I
925  CTG CGT GAT TCT CTT AGC GAT AAA CCT GCA AAA AAT ATT ATT
     GAC GCA CTA AGA GAA TCG CTA TTT GGA CGT TTT TTA TAA TAA
```

FIG. 26-2

```
  +1  L   L   I   G   D   G   M   G   D   S   E   I   T   A
463  TTG CTG ATT GGC GAT GGG ATG GGG GAC TCG GAA ATT ACT GCC
     AAC GAC TAA CCG CTA CCC TAC CCC CTG AGC CTT TAA TGA CGG

  +1  A   R   N   Y   A   E   G   A   G   G   F   F   K   G
505  GCA CGT AAT TAT GCC GAA GGT GCG GGC GGC TTT TTT AAA GGT
     CGT GCA TTA ATA CGG CTT CCA CGC CCG CCG AAA AAA TTT CCA

  +1  I   D   A   L   P   L   T   G   Q   Y   T   H   Y   A
1051 ATA GAT GCC TTA CCG CTT ACC GGG CAA TAC ACT CAC TAT GCG
     TAT CTA CGG AAT GGC GAA TGG CCC GTT ATG TGA GTG ATA CGC

  +1  L   N   K   K   T   G   K   P   D   Y   V   T   D   S
1093 CTG AAT AAA AAA ACC GGC AAA CCG GAC TAC GTC ACC GAC TCG
     GAC TTA TTT TTT TGG CCG TTT GGC CTG ATG CAG TGG CTG AGC

  +1  A   A   S   A   T   A   W   S   T   G   V   K   T   Y
1135 GCT GCA TCA GCA ACC GCC TGG TCA ACC GGT GTC AAA ACC TAT
     CGA CGT AGT CGT TGG CGG ACC AGT TGG CCA CAG TTT TGG ATA

  +1  N   G   A   L   G   V   D   I   H   E   K   D   H   P
1177 AAC GGC GCG CTG GGC GTC GAT ATT CAC GAA AAA GAT CAC CCA
     TTG CCG CGC GAC CCG CAG CTA TAA GTG CTT TTT CTA GTG GGT

  +1  T   I   L   E   M   A   K   A   A   G   L   A   T   G
1219 ACG ATT CTG GAA ATG GCA AAA GCC GCA GGT CTG GCG ACC GGT
     TGC TAA GAC CTT TAC CGT TTT CGG CGT CCA GAC CGC TGG CCA

  +1  N   V   S   T   A   E   L   Q   D   A   T   P   A   A
1261 AAC GTT TCT ACC GCA GAG TTG CAG GAT GCC ACG CCC GCT GCG
     TTG CAA AGA TGG CGT CTC AAC GTC CTA CGG TGC GGG CGA CGC

  +1  L   V   A   H   V   T   S   R   K   C   Y   G   P   S
1303 CTG GTG GCA CAT GTG ACC TCG CGC AAA TGC TAC GGT CCG AGC
     GAC CAC CGT GTA CAC TGG AGC GCG TTT ACG ATG CCA GGC TCG

  +1  A   T   S   E   K   C   P   G   N   A   L   E   K   G
1345 GCG ACC AGT GAA AAA TGT CCG GGT AAC GCT CTG GAA AAA GGC
     CGC TGG TCA CTT TTT ACA GGC CCA TTG CGA GAC CTT TTT CCG

  +1  G   K   G   S   I   T   E   Q   L   L   N   A   R   A
1387 GGA AAA GGA TCG ATT ACC GAA CAG CTG CTT AAC GCT CGT GCC
     CCT TTT CCT AGC TAA TGG CTT GTC GAC GAA TTG CGA GCA CGG

  +1  D   V   T   L   G   G   G   A   K   T   F   A   E   T
1429 GAC GTT ACG CTT GGC GGC GGC GCA AAA ACC TTT GCT GAA ACG
     CTG CAA TGC GAA CCG CCG CCG CGT TTT TGG AAA CGA CTT TGC
```

FIG. 26-3

```
   +1  A   T   A   G   E   W   Q   G   K   T   L   R   E   Q
1471  GCA ACC GCT GGT GAA TGG CAG GGA AAA ACG CTG CGT GAA CAG
      CGT TGG CGA CCA CTT ACC GTC CCT TTT TGC GAC GCA CTT GTC

   +1  A   Q   A   R   G   Y   Q   L   V   S   D   A   A   S
1513  GCA CAG GCG CGT GGT TAT CAG TTG GTG AGC GAT GCT GCC TCA
      CGT GTC CGC GCA CCA ATA GTC AAC CAC TCG CTA CGA CGG AGT

   +1  L   N   S   V   T   E   A   N   Q   Q   K   P   L   L
1555  CTG AAT TCG GTG ACG GAA GCG AAT CAG CAA AAA CCC CTG CTT
      GAC TTA AGC CAC TGC CTT CGC TTA GTC GTT TTT GGG GAC GAA

   +1  G   L   F   A   D   G   N   M   P   V   R   W   L   G
1177  GGC CTG TTT GCT GAC GGC AAT ATG CCA GTG CGC TGG CTA GGA
      CCG GAC AAA CGA CTG CCG TTA TAC GGT CAC GCG ACC GAT CCT

   +1  P   K   A   T   Y   H   G   N   I   D   K   P   A   V
1639  CCG AAA GCA ACG TAC CAT GGC AAT ATC GAT AAG CCC GCA GTC
      GGC TTT CGT TGC ATG GTA CCG TTA TAG CTA TTC GGG CGT CAG

   +1  T   C   T   P   N   P   Q   R   N   D   S   V   P   T
1681  ACC TGT ACG CCA AAT CCG CAA CGT AAT GAC AGT GTA CCA ACC
      TGG ACA TGC GGT TTA GGC GTT GCA TTA CTG TCA CAT GGT TGG

   +1  L   A   Q   M   T   D   K   A   I   E   L   L   S   K
1723  CTG GCG CAG ATG ACC GAC AAA GCC ATT GAA TTG TTG AGT AAA
      GAC CGC GTC TAC TGG CTG TTT CGG TAA CTT AAC AAC TCA TTT

   +1  N   E   K   G   F   F   L   Q   V   E   G   A   S   I
1765  AAT GAG AAA GGC TTT TTC CTG CAA GTT GAA GGT GCG TCA ATC
      TTA CTC TTT CCG AAA AAG GAC GTT CAA CTT CCA CGC AGT TAG

   +1  D   K   Q   D   H   A   A   N   P   C   G   Q   I   G
1807  GAT AAA CAG GAT CAT GCT GCG AAT CCT TGT GGG CAA ATT GGC
      CTA TTT GTC CTA GTA CGA CGC TTA GGA ACA CCC GTT TAA CCG

   +1  E   T   V   D   L   D   E   A   V   Q   R   A   L   E
1849  GAG ACG GTC GAT CTC GAT GAA GCC GTA CAA CGG GCG CTG GAA
      CTC TGC CAG CTA GAG CTA CTT CGG CAT GTT GCC CGC GAC CTT

   +1  F   A   K   K   E   G   N   T   L   V   I   V   T   A
1891  TTC GCT AAA AAG GAG GGT AAC ACG CTG GTC ATA GTC ACC GCT
      AAG CGA TTT TTC CTC CCA TTG TGC GAC CAG TAT CAG TGG CGA

   +1  D   H   A   H   A   S   Q   I   V   A   P   D   T   K
1933  GAT CAC GCC CAC GCC AGC CAG ATT GTT GCG CCG GAT ACC AAA
      CTA GTG CGG GTG CGG TCG GTC TAA CAA CGC GGC CTA TGG TTT

   +1  A   P   G   L   T   Q   A   L   N   T   K   D   G   A
1975  GCT CCG GGC CTC ACC CAG GCG CTA AAT ACC AAA GAT GGC GCA
      CGA GGC CCG GAG TGG GTC CGC GAT TTA TGG TTT CTA CCG CGT
```

FIG. 26-4

```
   +1  V   M   V   M   S   Y   G   N   S   E   E   D   S   Q
2017  GTG ATG GTG ATG AGT TAC GGG AAC TCC GAA GAG GAT TCA CAA
      CAC TAC CAC TAC TCA ATG CCC TTG AGG CTT CTC CTA AGT GTT

   +1  E   H   T   G   S   Q   L   R   I   A   A   Y   G   P
2059  GAA CAT ACC GGC AGT CAG TTG CGT ATT GCG GCG TAT GGC CCG
      CTT GTA TGG CCG TCA GTC AAC GCA TAA CGC CGC ATA CCG GGC

   +1  H   A   A   N   V   V   G   L   T   D   Q   T   D   L
2101  CAT GCC GCC AAT GTT GTT GGA CTG ACC GAC CAG ACC GAT CTC
      GTA CGG CGG TTA CAA CAA CCT GAC TGG CTG GTC TGG CTA GAG

                                                    |His tag
   +1  F   Y   T   M   K   A   A   L   G   D   I   A   H   H
2143  TTC TAC ACC ATG AAA GCC GCT CTG GGG GAT ATC|GCA CAC CAT
      AAG ATG TGG TAC TTT CGG CGA GAC CCC CTA TAG|CGT GTG GTA

   +1  H   H   H   H   *
2185  CAC CAT CAC CAT TAA
      GTG GTA GTG GTA ATT
```

FIG. 26-5

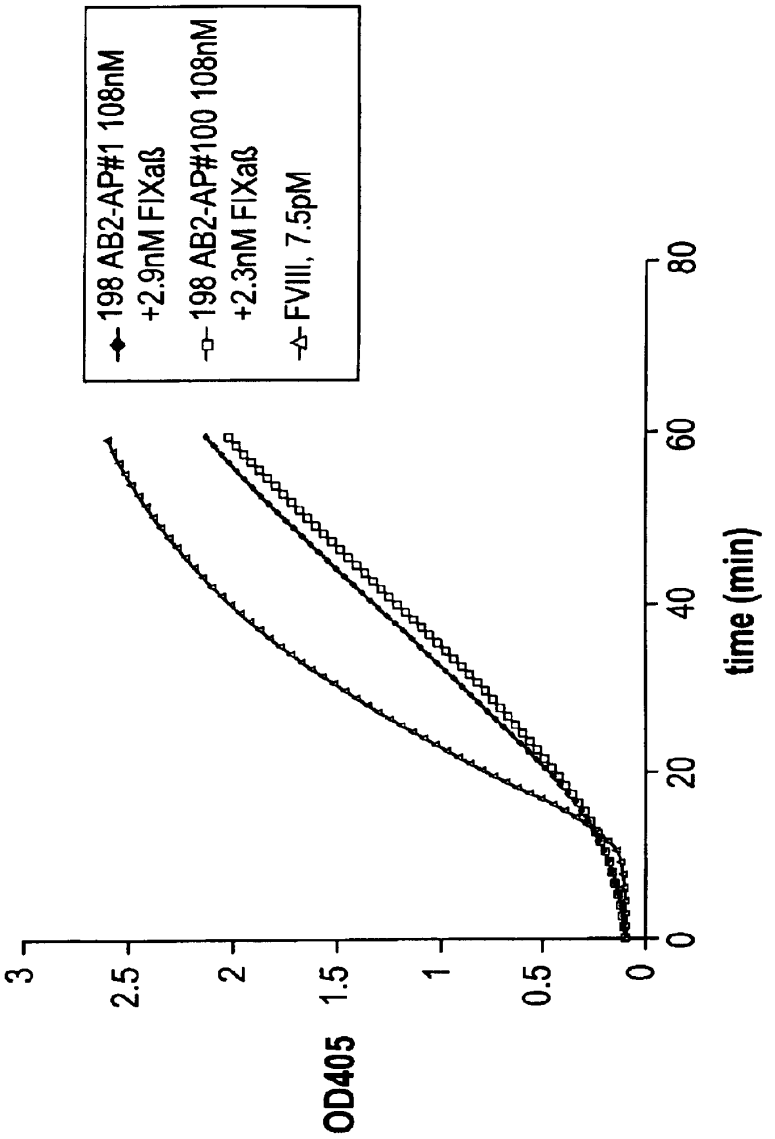

198 AB2-AP#1 108nM
+2.9nM FIXaß
198 AB2-AP#100 108nM
+2.3nM FIXaß
FVIII, 7.5pM
OD405
time (min)
0
0.5
1
1.5
2
2.5
3
0
20
40
60
80

FIG. 27

PelB-Leader

```
+1   M   K   Y   L   L   P   T   A   A   A   G   L   L   L   L
1   ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC
    TAC TTT ATG GAT AAC GGA TGC CGT CGG CGA CCT AAC AAT AAT GAG

+1   A   A   Q   P   A   M   A   E   V   K   L   V   E   S   G
46  GCG GCC CAG CCG GCC ATG GCG GAG GTG AAG CTG GTG GAG TCT GGG
    CGC CGG GTC GGC CGG TAC CGC CTC CAC TTC GAC CAC CTC AGA CCC

+1   G   G   L   V   K   P   G   G   S   L   K   L   S   C   A
91  GGA GGC TTA GTG AAG CCT GGA GGG TCC CTG AAA CTC TCC TGT GCA
    CCT CCG AAT CAC TTC GGA CCT CCC AGG GAC TTT GAG AGG ACA CGT

+1   A   S   G   F   T   F   S   S   Y   T   M   S   W   V   R
136 GCC TCT GGA TTC ACT TTC AGT AGC TAT ACC ATG TCT TGG GTT CGC
    CGG AGA CCT AAG TGA AAG TCA TCG ATA TGG TAC AGA ACC CAA GCG

+1   Q   T   P   E   K   R   L   E   W   V   A   T   I   S   S
181 CAG ACT CCG GAG AAG AGG CTG GAG TGG GTC GCA ACC ATT AGT AGT
    GTC TGA GGC CTC TTC TCC GAC CTC ACC CAG CGT TGG TAA TCA TCA

+1   G   G   S   S   T   Y   Y   P   D   S   V   K   G   R   F
226 GGN GGT AGT TCC ACC TAC TAT CCA GAC AGT GTG AAG GGC CGA TTC
    CCN CCA TCA AGG TGG ATG ATA GGT CTG TCA CAC TTC CCG GCT AAG

+1   T   I   S   R   D   N   A   K   N   T   L   Y   L   Q   M
271 ACC ATC TCC AGA GAC AAT GCC AAG AAC ACC CTG TAC CTG CAA ATG
    TGG TAG AGG TCT CTG TTA CGG TTC TTG TGG GAC ATG GAC GTT TAC

+1   S   S   L   R   S   E   D   T   A   M   Y   Y   C   T   R
316 AGC AGT CTG AGG TCT GAG GAC ACA GCC ATG TAT TAC TGT ACA AGA
    TCG TCA GAC TCC AGA CTC CTG TGT CGG TAC ATA ATG ACA TGT TCT

+1   E   G   G   G   F   T   V   N   W   Y   F   D   V   W   G
361 GAG GGG GGT GGT TTC ACC GTC AAC TGG TAC TTC GAT GTC TGG GGC
    CTC CCC CCA CCA AAG TGG CAG TTG ACC ATG AAG CTA CAG ACC CCG

                                                Linker
+1   A   G   T   S   V   T   V   S   S   G   G   G   G   S   G
406 GCA GGA ACC TCA GTC ACC GTC TCC TCA GGT GGA GGC GGT TCA GGT
    CGT CCT TGG AGT CAG TGG CAG AGG AGT CCA CCT CCG CCA AGT CCA

                                                VL
+1   G   R   A   S   G   G   G   G   S   D   I   V   L   T   Q
451 GGG CGC GCC TCT GGC GGT GGC GGA TCG GAC ATT GTG CTG ACA CAG
    CCC GCG CGG AGA CCG CCA CCG CCT AGC CTG TAA CAC GAC TGT GTC
```

FIG. 28-1

```
  +1  X   P   A   S   L   A   V   S   L   G   Q   R   A   T   I
496  TNT CCA GCT TCT TTG GCT GTG TCT CTA GGG CAG AGG GCC ACC ATA
     ANA GGT CGA AGA AAC CGA CAC AGA GAT CCC GTC TCC CGG TGG TAT

  +1  S   C   R   A   S   E   S   V   D   S   Y   G   Y   N   F
541  TCN TGC AGA GCC AGT GAA AGT GTT GAT AGT TAT GGC TAT AAT TTT
     AGN ACG TCT CGG TCA CTT TCA CAA CTA TCA ATA CCG ATA TTA AAA

  +1  M   H   W   Y   Q   Q   I   P   G   Q   P   P   K   L   L
586  ATG CAC TGG TAT CAG CAG ATA CCA GGA CAG CCA CCC AAA CTC CTC
     TAC GTG ACC ATA GTC GTC TAT GGT CCT GTC GGT GGG TTT GAG GAG

  +1  I   Y   R   A   S   N   L   E   S   G   I   P   A   R   F
631  ATC TAT CGT GCA TCC AAC CTA GAG TCT GGG ATC CCT GCC AGG TTC
     TAG ATA GCA CGT AGG TTG GAT CTC AGA CCC TAG GGA CGG TCC AAG

  +1  S   G   S   G   S   R   T   D   F   T   L   T   I   N   P
676  AGT GGC AGT GGG TCT AGG ACA GAC TTC ACC CTC ACC ATT AAT CCT
     TCA CCG TCA CCC AGA TCC TGT CTG AAG TGG GAG TGG TAA TTA GGA

  +1  V   E   A   D   D   V   A   T   Y   Y   C   Q   Q   S   N
721  GTG GAG GCT GAT GAT GTT GCA ACC TAT TAC TGT CAG CAA AGT AAT
     CAC CTC CGA CTA CTA CAA CGT TGG ATA ATG ACA GTC GTT TCA TTA

  +1  E   D   P   L   T   F   G   T   G   T   R   L   E   I   K
766  GAG GAT CCG CTC ACG TTC GGT ACT GGG ACC AGA CTG GAA ATA AAA
     CTC CTA GGC GAG TGC AAG CCA TGA CCC TGG TCT GAC CTT TAT TTT

          Spacer    Hinge                                  Helix

  +1  R   A   A   A   P   K   P   S   T   P   P   G   S   S   R
811  CGG GCG GCC GCA CCG AAG CCT TCC ACT CCG CCC GGG TCT TCC CGT
     GCC CGC CGG CGT GGC TTC GGA AGG TGA GGC GGG CCC AGA AGG GCA

  +1  M   K   Q   L   E   D   K   V   E   E   L   L   S   K   N
856  ATG AAA CAG CTG GAA GAC AAA GTA GAG GAG CTC CTT AGC AAG AAC
     TAC TTT GTC GAC CTT CTG TTT CAT CTC CTC GAG GAA TCG TTC TTG

  +1  Y   H   L   E   N   E   V   A   R   L   K   K   L   V   G
901  TAC CAT CTA GAA AAC GAG GTA GCT CGT CTG AAA AAG CTT GTT GGT
     ATG GTA GAT CTT TTG CTC CAT CGA GCA GAC TTT TTC GAA CAA CCA

          Spacer     His-tag
  +1  E   R   G   G   H   H   H   H   H   H   *
946  GAA CGT GGT GGT CAC CAT CAC CAT CAC CAT TAA
     CTT GCA CCA CCA GTG GTA GTG GTA GTG GTA ATT
```

FIG. 28-2

PelB-Leader

```
+1   M   K   Y   L   L   P   T   A   A   A   G   L   L   L
1   ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA
    TAC TTT ATG GAT AAC GGA TGC CGT CGG CGA CCT AAC AAT AAT

                                                VH
+1   L   A   A   Q   P   A   M   A | E   V   Q   L   Q   Q
43  CTC GCG GCC CAG CCG GCC ATG GCC|GAG GTT CAG CTT CAG CAG
    GAG CGC CGG GTC GGC CGG TAC CGG|CTC CAA GTC GAA GTC GTC

+1   S   G   P   E   L   V   K   P   G   A   S   V   K   I
85  TCT GGA CCT GAG CTG GTG AAG CCC GGG GCC TCA GTG AAG ATT
    AGA CCT GGA CTC GAC CAC TTC GGG CCC CGG AGT CAC TTC TAA

+1   S   C   K   A   S   G   Y   A   F   S   S   S   W   M
127 TCC TGC AAA GCT TCT GGC TAC GCA TTC AGT AGC TCT TGG ATG
    AGG ACG TTT CGA AGA CCG ATG CGT AAG TCA TCG AGA ACC TAC

+1   N   W   V   K   Q   R   P   G   Q   G   L   E   W   I
169 AAC TGG GTG AAG CAG AGG CCT GGA CAG GGT CTT GAG TGG ATT
    TTG ACC CAC TTC GTC TCC GGA CCT GTC CCA GAA CTC ACC TAA

+1   G   R   I   Y   P   G   N   G   D   T   N   Y   N   G
211 GGA CGG ATT TAT CCT GGA AAT GGA GAT ACT AAC TAC AAT GGG
    CCT GCC TAA ATA GGA CCT TTA CCT CTA TGA TTG ATG TTA CCC

+1   K   F   K   G   K   A   T   L   T   A   D   K   S   S
253 AAG TTC AAG GGC AAG GCC ACA CTG ACT GCA GAC AAA TCC TCC
    TTC AAG TTC CCG TTC CGG TGT GAC TGA CGT CTG TTT AGG AGG

+1   S   T   A   Y   M   Q   L   S   S   L   T   S   V   D
295 AGC ACA GCC TAC ATG CAG CTC AGC AGC CTG ACC TCT GTG GAC
    TCG TGT CGG ATG TAC GTC GAG TCG TCG GAC TGG AGA CAC CTG

+1   S   A   V   Y   F   C   A   D   G   N   V   Y   Y   Y
337 TCT GCG GTC TAT TTC TGT GCA GAT GGT AAC GTA TAT TAC TAT
    AGA CGC CAG ATA AAG ACA CGT CTA CCA TTG CAT ATA ATG ATA

+1   A   M   D   Y   W   G   Q   G   T   S   V   T   V   S
379 GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC
    CGA TAC CTG ATG ACC CCA GTT CCT TGG AGT CAG TGG CAG AGG

          Linker
+1   S | G   G   G   G   S   G   G   R   A   S   G   G   G
421 TCA|GGT GGA GGC GGT TCA GGT GGG CGC GCC TCT GGC GGT GGC
    AGT|CCA CCT CCG CCA AGT CCA CCC GCG CGG AGA CCG CCA CCG

      |   VL
+1   G   S | Q   I   V   L   T   Q   S   P   A   S   L   A
463 GGA TCG|CAA ATT GTT CTC ACC CAG TCT CCT GCT TCC TTA GCT
    CCT AGC|GTT TAA CAA GAG TGG GTC AGA GGA CGA AGG AAT CGA
```

FIG. 29-1

```
  +1  V   S   L   G   Q   R   A   T   I   S   C   R   A   S
505  GTA TCT CTG GGG CAG AGG GCC ACC ATC TCA TGC AGG GCC AGC
     CAT AGA GAC CCC GTC TCC CGG TGG TAG AGT ACG TCC CGG TCG

  +1  K   S   V   S   T   S   G   Y   S   Y   M   H   W   Y
547  AAA AGT GTC AGT ACA TCT GGC TAT AGT TAT ATG CAC TGG TAC
     TTT TCA CAG TCA TGT AGA CCG ATA TCA ATA TAC GTG ACC ATG

  +1  Q   Q   K   P   G   Q   P   P   K   L   L   I   Y   L
589  CAA CAG AAA CCA GGA CAG CCA CCC AAA CTC CTC ATC TAT CTT
     GTT GTC TTT GGT CCT GTC GGT GGG TTT GAG GAG TAG ATA GAA

  +1  A   S   N   L   E   S   G   V   P   A   R   F   S   G
631  GCA TCC AAC CTA GAA TCT GGG GTC CCT GCC AGG TTC AGT GGC
     CGT AGG TTG GAT CTT AGA CCC CAG GGA CGG TCC AAG TCA CCG

  +1  S   G   S   G   T   D   F   T   L   N   I   H   P   V
679  AGT GGG TCT GGG ACA GAC TTC ACC CTC AAC ATC CAT CCT GTG
     TCA CCC AGA CCC TGT CTG AAG TGG GAG TTG TAG GTA GGA CAC

  +1  E   E   E   D   A   A   T   Y   Y   C   Q   H   S   R
715  GAG GAG GAG GAT GCT GCA ACC TAT TAC TGT CAG CAC AGT AGG
     CTC CTC CTC CTA CGA CGT TGG ATA ATG ACA GTC GTG TCA TCC

  +1  E   L   P   R   T   F   G   G   G   T   K   L   E   I
757  GAG CTT CCT CGG ACG TTC GGT GGA GGC ACC AAG CTG GAA ATC
     CTC GAA GGA GCC TGC AAG CCA CCT CCG TGG TTC GAC CTT TAG

                 Spacer                Alkaline phosphatase
  +1  K   R  |  A   A   A   A  |  R   A   P   E   M   P   V   L
799  AAA CGG|GCG GCC GCA GCC|CGG GCA CCA GAA ATG CCT GTT CTG
     TTT GCC|CGC CGG CGT CGG|GCC CGT GGT CTT TAC GGA CAA GAC

  +1  E   N   R   A   A   Q   G   D   I   T   A   P   G   G
841  GAA AAC CGG GCT GCT CAG GGC GAT ATT ACT GCA CCC GGC GGT
     CTT TTG GCC CGA CGA GTC CCG CTA TAA TGA CGT GGG CCG CCA

  +1  A   R   R   L   T   G   D   Q   T   A   A   L   R   D
883  GCT CGC CGT TTA ACG GGT GAT CAG ACT GCC GCT CTG CGT GAT
     CGA GCG GCA AAT TGC CCA CTA GTC TGA CGG CGA GAC GCA CTA

  +1  S   L   S   D   K   P   A   K   N   I   I   L   L   I
925  TCT CTT AGC GAT AAA CCT GCA AAA AAT ATT ATT TTG CTG ATT
     AGA GAA TCG CTA TTT GGA CGT TTT TTA TAA TAA AAC GAC TAA


  +1  G   D   G   M   G   D   S   E   I   T   A   A   R   N
967  GGC GAT GGG ATG GGG GAC TCG GAA ATT ACT GCC GCA CGT AAT
     CCG CTA CCC TAC CCC CTG AGC CTT TAA TGA CGG CGT GCA TTA
```

FIG. 29-2

```
   +1  Y   A   E   G   A   G   G   F   F   K   G   I   D   A
1009  TAT GCC GAA GGT GCG GGC GGC TTT TTT AAA GGT ATA GAT GCC
      ATA CGG CTT CCA CGC CCG CCG AAA AAA TTT CCA TAT CTA CGG

   +1  L   P   L   T   G   Q   Y   T   H   Y   A   L   N   K
1051  TTA CCG CTT ACC GGG CAA TAC ACT CAC TAT GCG CTG AAT AAA
      AAT GGC GAA TGG CCC GTT ATG TGA GTG ATA CGC GAC TTA TTT

   +1  K   T   G   K   P   D   Y   V   T   D   S   A   A   S
1093  AAA ACC GGC AAA CCG GAC TAC GTC ACC GAC TCG GCT GCA TCA
      TTT TGG CCG TTT GGC CTG ATG CAG TGG CTG AGC CGA CGT AGT

   +1  A   T   A   W   S   T   G   V   K   T   Y   N   G   A
1135  GCA ACC GCC TGG TCA ACC GGT GTC AAA ACC TAT AAC GGC GCG
      CGT TGG CGG ACC AGT TGG CCA CAG TTT TGG ATA TTG CCG CGC

   +1  L   G   V   D   I   H   E   K   D   H   P   T   I   L
1177  CTG GGC GTC GAT ATT CAC GAA AAA GAT CAC CCA ACG ATT CTG
      GAC CCG CAG CTA TAA GTG CTT TTT CTA GTG GGT TGC TAA GAC

   +1  E   M   A   K   A   A   G   L   A   T   G   N   V   S
1219  GAA ATG GCA AAA GCC GCA GGT CTG GCG ACC GGT AAC GTT TCT
      CTT TAC CGT TTT CGG CGT CCA GAC CGC TGG CCA TTG CAA AGA

   +1  T   A   E   L   Q   D   A   T   P   A   A   L   V   A
1261  ACC GCA GAG TTG CAG GAT GCC ACG CCC GCT GCG CTG GTG GCA
      TGG CGT CTC AAC GTC CTA CGG TGC GGG CGA CGC GAC CAC CGT

   +1  H   V   T   S   R   K   C   Y   G   P   S   A   T   S
1303  CAT GTG ACC TCG CGC AAA TGC TAC GGT CCG AGC GCG ACC AGT
      GTA CAC TGG AGC GCG TTT ACG ATG CCA GGC TCG CGC TGG TCA

   +1  E   K   C   P   G   N   A   L   E   K   G   G   K   G
1345  GAA AAA TGT CCG GGT AAC GCT CTG GAA AAA GGC GGA AAA GGA
      CTT TTT ACA GGC CCA TTG CGA GAC CTT TTT CCG CCT TTT CCT

   +1  S   I   T   E   Q   L   L   N   A   R   A   D   V   T
1387  TCG ATT ACC GAA CAG CTG CTT AAC GCT CGT GCC GAC GTT ACG
      AGC TAA TGG CTT GTC GAC GAA TTG CGA GCA CGG CTG CAA TGC

   +1  L   G   G   G   A   K   T   F   A   E   T   A   T   A
1429  CTT GGC GGC GGC GCA AAA ACC TTT GCT GAA ACG GCA ACC GCT
      GAA CCG CCG CCG CGT TTT TGG AAA CGA CTT TGC CGT TGG CGA

   +1  G   E   W   Q   G   K   T   L   R   E   Q   A   Q   A
1471  GGT GAA TGG CAG GGA AAA ACG CTG CGT GAA CAG GCA CAG GCG
      CCA CTT ACC GTC CCT TTT TGC GAC GCA CTT GTC CGT GTC CGC
```

FIG. 29-3

```
   +1  R   G   Y   Q   L   V   S   D   A   A   S   L   N   S
1513  CGT GGT TAT CAG TTG GTG AGC GAT GCT GCC TCA CTG AAT TCG
      GCA CCA ATA GTC AAC CAC TCG CTA CGA CGG AGT GAC TTA AGC

   +1  V   T   E   A   N   Q   Q   K   P   L   L   G   L   F
1555  GTG ACG GAA GCG AAT CAG CAA AAA CCC CTG CTT GGC CTG TTT
      CAC TGC CTT CGC TTA GTC GTT TTT GGG GAC GAA CCG GAC AAA

   +1  A   D   G   N   M   P   V   R   W   L   G   P   K   A
1597  GCT GAC GGC AAT ATG CCA GTG CGC TGG CTA GGA CCG AAA GCA
      CGA CTG CCG TTA TAC GGT CAC GCG ACC GAT CCT GGC TTT CGT

   +1  T   Y   H   G   N   I   D   K   P   A   V   T   C   T
1639  ACG TAC CAT GGC AAT ATC GAT AAG CCC GCA GTC ACC TGT ACG
      TGC ATG GTA CCG TTA TAG CTA TTC GGG CGT CAG TGG ACA TGC

   +1  P   N   P   Q   R   N   D   S   V   P   T   L   A   Q
1681  CCA AAT CCG CAA CGT AAT GAC AGT GTA CCA ACC CTG GCG CAG
      GGT TTA GGC GTT GCA TTA CTG TCA CAT GGT TGG GAC CGC GTC

   +1  M   T   D   K   A   I   E   L   L   S   K   N   E   K
1723  ATG ACC GAC AAA GCC ATT GAA TTG TTG AGT AAA AAT GAG AAA
      TAC TGG CTG TTT CGG TAA CTT AAC AAC TCA TTT TTA CTC TTT

   +1  G   F   F   L   Q   V   E   G   A   S   I   D   K   Q
1765  GGC TTT TTC CTG CAA GTT GAA GGT GCG TCA ATC GAT AAA CAG
      CCG AAA AAG GAC GTT CAA CTT CCA CGC AGT TAG CTA TTT GTC

   +1  D   H   A   A   N   P   C   G   Q   I   G   E   T   V
1807  GAT CAT GCT GCG AAT CCT TGT GGG CAA ATT GGC GAG ACG GTC
      CTA GTA CGA CGC TTA GGA ACA CCC GTT TAA CCG CTC TGC CAG

   +1  D   L   D   E   A   V   Q   R   A   L   E   F   A   K
1849  GAT CTC GAT GAA GCC GTA CAA CGG GCG CTG GAA TTC GCT AAA
      CTA GAG CTA CTT CGG CAT GTT GCC CGC GAC CTT AAG CGA TTT

   +1  K   E   G   N   T   L   V   I   V   T   A   D   H   A
1891  AAG GAG GGT AAC ACG CTG GTC ATA GTC ACC GCT GAT CAC GCC
      TTC CTC CCA TTG TGC GAC CAG TAT CAG TGG CGA CTA GTG CGG

   +1  H   A   S   Q   I   V   A   P   D   T   K   A   P   G
1933  CAC GCC AGC CAG ATT GTT GCG CCG GAT ACC AAA GCT CCG GGC
      GTG CGG TCG GTC TAA CAA CGC GGC CTA TGG TTT CGA GGC CCG

   +1  L   T   Q   A   L   N   T   K   D   G   A   V   M   V
1975  CTC ACC CAG GCG CTA AAT ACC AAA GAT GGC GCA GTG ATG GTG
      GAG TGG GTC CGC GAT TTA TGG TTT CTA CCG CGT CAC TAC CAC
```

FIG. 29-4

```
   +1  M   S   Y   G   N   S   E   E   D   S   Q   E   H   T
2017   ATG AGT TAC GGG AAC TCC GAA GAG GAT TCA CAA GAA CAT ACC
       TAC TCA ATG CCC TTG AGG CTT CTC CTA AGT GTT CTT GTA TGG

   +1  G   S   Q   L   R   I   A   A   Y   G   P   H   A   A
2059   GGC AGT CAG TTG CGT ATT GCG GCG TAT GGC CCG CAT GCC GCC
       CCG TCA GTC AAC GCA TAA CGC CGC ATA CCG GGC GTA CGG CGG

   +1  N   V   V   G   L   T   D   Q   T   D   L   F   Y   T
2101   AAT GTT GTT GGA CTG ACC GAC CAG ACC GAT CTC TTC TAC ACC
       TTA CAA CAA CCT GAC TGG CTG GTC TGG CTA GAG AAG ATG TGG

                                                  His tag
   +1  M   K   A   A   L   G   D   I   A  |H   H   H   H   H
2143   ATG AAA GCC GCT CTG GGG GAT ATC GCA |CAC CAT CAC CAT CAC
       TAC TTT CGG CGA GAC CCC CTA TAG CGT |GTG GTA GTG GTA GTG

   +1  H   *
2185   CAT TAA
       GTA ATT
```

FIG. 29-5

PelB-Leader

```
+1   M   K   Y   L   L   P   T   A   A   A   G   L   L   L   L
1   ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC
    TAC TTT ATG GAT AAC GGA TGC CGT CGG CGA CCT AAC AAT AAT GAG

                                          VH
+1   A   A   Q   P   A   M   A | E   V   Q   L   Q   Q   S   G
46  GCG GCC CAG CCG GCC ATG GCG|GAG GTT CAG CTT CAG CAG TCT GGA
    CGC CGG GTC GGC CGG TAC CGC|CTC CAA GTC GAA GTC GTC AGA CCT

+1   P   E   L   V   K   P   G   A   S   V   K   I   S   C   K
91  CCT GAG CTG GTG AAG CCC GGG GCC TCA GTG AAG ATT TCC TGC AAA
    GGA CTC GAC CAC TTC GGG CCC CGG AGT CAC TTC TAA AGG ACG TTT

+1   A   S   G   Y   A   F   S   S   S   W   M   N   W   V   K
136 GCT TCT GGC TAC GCA TTC AGT AGC TCT TGG ATG AAC TGG GTG AAG
    CGA AGA CCG ATG CGT AAG TCA TCG AGA ACC TAC TTG ACC CAC TTC

+1   Q   R   P   G   Q   G   L   E   W   I   G   R   I   Y   P
181 CAG AGG CCT GGA CAG GGT CTT GAG TGG ATT GGA CGG ATT TAT CCT
    GTC TCC GGA CCT GTC CCA GAA CTC ACC TAA CCT GCC TAA ATA GGA

+1   G   N   G   D   T   N   Y   N   G   K   F   K   G   K   A
226 GGA AAT GGA GAT ACT AAC TAC AAT GGG AAG TTC AAG GGC AAG GCC
    CCT TTA CCT CTA TGA TTG ATG TTA CCC TTC AAG TTC CCG TTC CGG

+1   T   L   T   A   D   K   S   S   S   T   A   Y   M   Q   L
271 ACA CTG ACT GCA GAC AAA TCC TCC AGC ACA GCC TAC ATG CAG CTC
    TGT GAC TGA CGT CTG TTT AGG AGG TCG TGT CGG ATG TAC GTC GAG

+1   S   S   L   T   S   V   D   S   A   V   Y   F   C   A   D
316 AGC AGC CTG ACC TCT GTG GAC TCT GCG GTC TAT TTC TGT GCA GAT
    TCG TCG GAC TGG AGA CAC CTG AGA CGC CAG ATA AAG ACA CGT CTA

+1   G   N   V   Y   Y   Y   A   M   D   Y   W   G   Q   G   T
361 GGT AAC GTA TAT TAC TAT GCT ATG GAC TAC TGG GGT CAA GGA ACC
    CCA TTG CAT ATA ATG ATA CGA TAC CTG ATG ACC CCA GTT CCT TGG

                                        Linker
+1   S   V   T   V   S   S | G   G   G   G   S   G   G   R   A
406 TCA GTC ACC GTC TCC TCA|GGT GGA GGC GGT TCA GGT GGG CGC GCC
    AGT CAG TGG CAG AGG AGT|CCA CCT CCG CCA AGT CCA CCC GCG CGG
```

FIG. 30-1

```
                                        |VL
  +1  S   G   G   G   G   S |Q   I   V   L   T   Q   S   P   A
451  TCT GGC GGT GGC GGA TCG|CAA ATT GTT CTC ACC CAG TCT CCT GCT
     AGA CCG CCA CCG CCT AGC|GTT TAA CAA GAG TGG GTC AGA GGA CGA

  +1  S   L   A   V   S   L   G   Q   R   A   T   I   S   C   R
496  TCC TTA GCT GTA TCT CTG GGG CAG AGG GCC ACC ATC TCA TGC AGG
     AGG AAT CGA CAT AGA GAC CCC GTC TCC CGG TGG TAG AGT ACG TCC


  +1  A   S   K   S   V   S   T   S   G   Y   S   Y   M   H   W
541  GCC AGC AAA AGT GTC AGT ACA TCT GGC TAT AGT TAT ATG CAC TGG
     CGG TCG TTT TCA CAG TCA TGT AGA CCG ATA TCA ATA TAC GTG ACC

  +1  Y   Q   Q   K   P   G   Q   P   P   K   L   L   I   Y   L
586  TAC CAA CAG AAA CCA GGA CAG CCA CCC AAA CTC CTC ATC TAT CTT
     ATG GTT GTC TTT GGT CCT GTC GGT GGG TTT GAG GAG TAG ATA GAA

  +1  A   S   N   L   E   S   G   V   P   A   R   F   S   G   S
631  GCA TCC AAC CTA GAA TCT GGG GTC CCT GCC AGG TTC AGT GGC AGT
     CGT AGG TTG GAT CTT AGA CCC CAG GGA CGG TCC AAG TCA CCG TCA

  +1  G   S   G   T   D   F   T   L   N   I   H   P   V   E   E
676  GGG TCT GGG ACA GAC TTC ACC CTC AAC ATC CAT CCT GTG GAG GAG
     CCC AGA CCC TGT CTG AAG TGG GAG TTG TAG GTA GGA CAC CTC CTC

  +1  E   D   A   A   T   Y   Y   C   Q   H   S   R   E   L   P
721  GAG GAT GCT GCA ACC TAT TAC TGT CAG CAC AGT AGG GAG CTT CCT
     CTC CTA CGA CGT TGG ATA ATG ACA GTC GTG TCA TCC CTC GAA GGA

                                                        |Spacer
  +1  R   T   F   G   G   G   T   K   L   E   I   K   R|A   A
766  CGG ACG TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA CGG|GCG GCC
     GCC TGC AAG CCA CCT CCG TGG TTC GAC CTT TAG TTT GCC|CGC CGG

       |Hinge                                          |Helix
  +1  A|P   K   P   S   T   P   P   G   S   S|R   M   K   Q
811  GCA|CCG AAG CCT TCC ACT CCG CCC GGG TCT TCC|CGT ATG AAA CAG
     CGT|GGC TTC GGA AGG TGA GGC GGG CCC AGA AGG|GCA TAC TTT GTC

  +1  L   E   D   K   V   E   E   L   L   S   K   N   Y   H   L
856  CTG GAA GAC AAA GTA GAG GAG CTC CTT AGC AAG AAC TAC CAT CTA
     GAC CTT CTG TTT CAT CTC CTC GAG GAA TCG TTC TTG ATG GTA GAT

  +1  E   N   E   V   A   R   L   K   K   L   V   G   E   R|G
901  GAA AAC GAG GTA GCT CGT CTG AAA AAG CTT GTT GGT GAA CGT|GGT
     CTT TTG CTC CAT CGA GCA GAC TTT TTC GAA CAA CCA CTT GCA|CCA
```

FIG. 30-2

	Spacer	His-tag						
+1	G	H	H	H	H	H	H	*
946	GGT	CAC	CAT	CAC	CAT	CAC	CAT	TAA
	CCA	GTG	GTA	GTG	GTA	GTG	GTA	ATT

FIG. 30-3

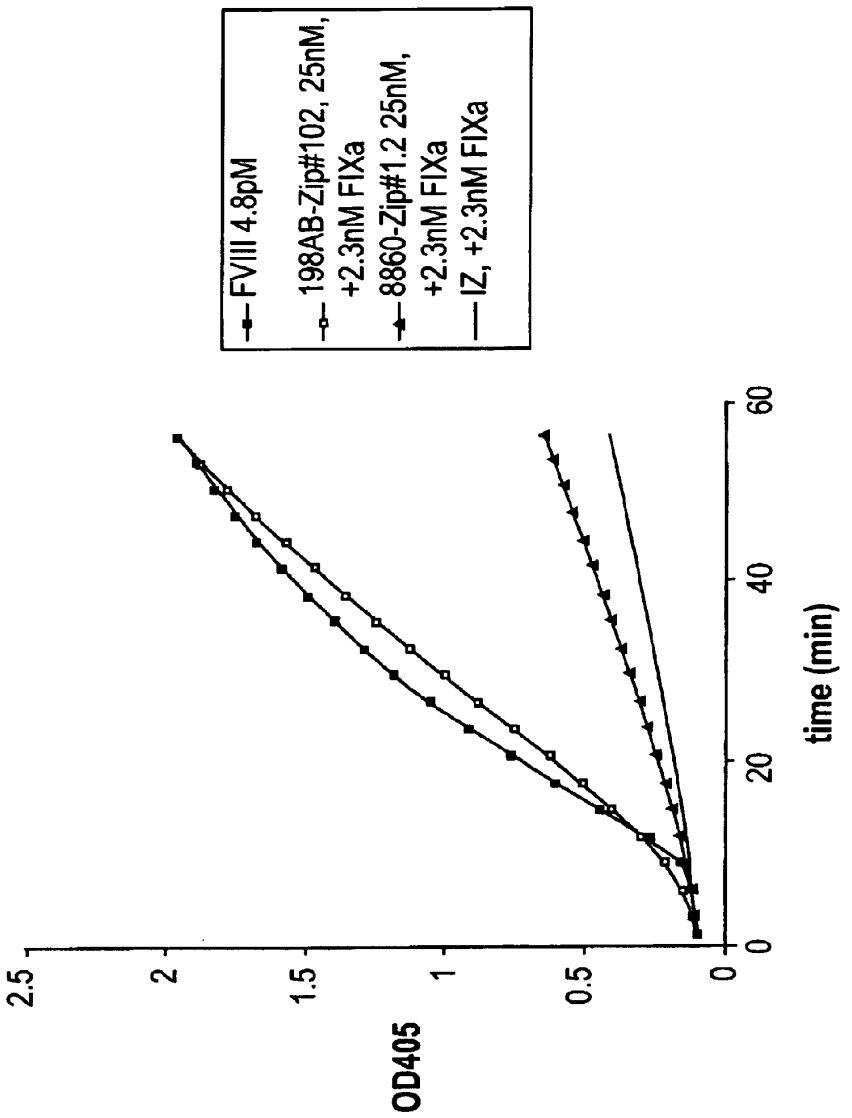

FVIII 4.8pM
198AB-Zip#102, 25nM, +2.3nM FIXa
8860-Zip#1.2 25nM, +2.3nM FIXa
IZ, +2.3nM FIXa
OD405
2.5
2
1.5
1
0.5
0
0
20
40
60
time (min)

FIG. 31

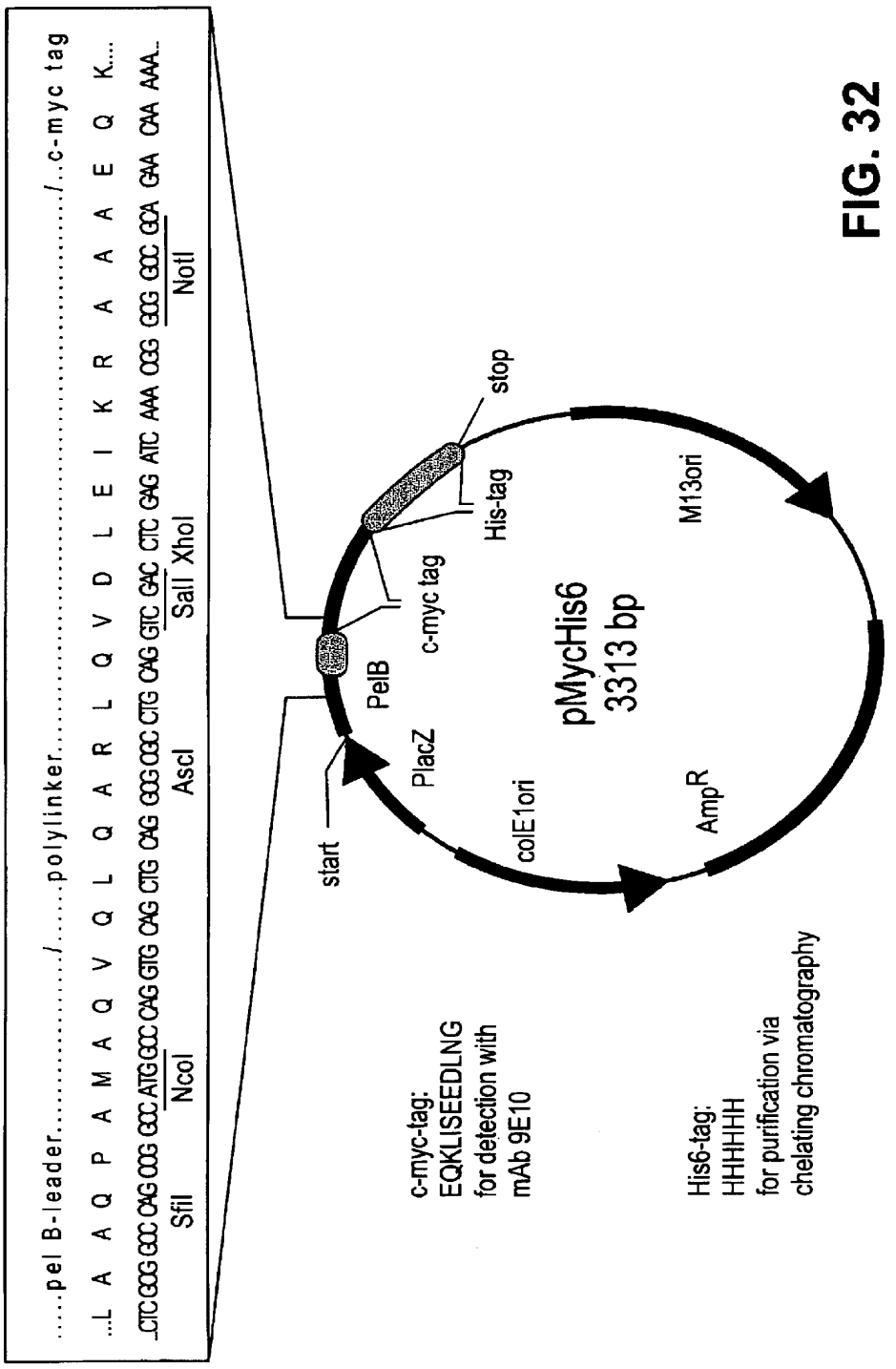

.....pel B-leader.....polylinker.....c-myc tag
...L A A Q P A M A Q V Q L Q A R L Q V D L E I K R A A A E Q K....
CTC GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG CAG GCG GCC CTG CAG GTC GAC CTC GAG ATC AAA CGG GCC GCA GAA CAA AAA
SfiI NcoI AscI SalI XhoI NotI
start
PelB
c-myc tag
His-tag
stop
PlacZ
colE1ori
pMycHis6
3313 bp
M13ori
AmpR
c-myc-tag:
EQKLISEEDLNG
for detection with
mAb 9E10
His6-tag:
HHHHHH
for purification via
chelating chromatography

FIG. 32

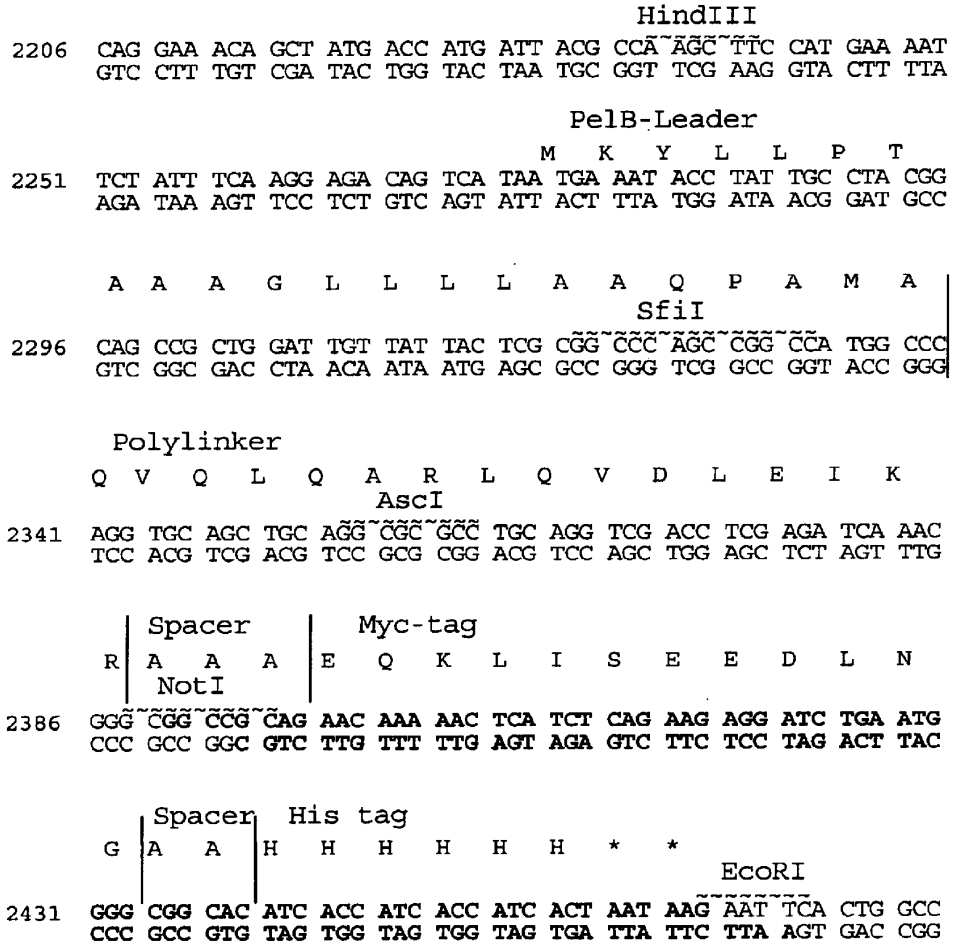

HindIII
2206 CAG GAA ACA GCT ATG ACC ATG ATT ACG CCA AGC TTC CAT GAA AAT
GTC CTT TGT CGA TAC TGG TAC TAA TGC GGT TCG AAG GTA CTT TTA
PelB-Leader
M K Y L L P T
2251 TCT ATT TCA AGG AGA CAG TCA TAA TGA AAT ACC TAT TGC CTA CGG
AGA TAA AGT TCC TCT GTC AGT ATT ACT TTA TGG ATA ACG GAT GCC
A A A G L L L L A A Q P A M A
SfiI
2296 CAG CCG CTG GAT TGT TAT TAC TCG CGG CCC AGC CGG CCA TGG CCC
GTC GGC GAC CTA ACA ATA ATG AGC GCC GGG TCG GCC GGT ACC GGG
Polylinker
Q V Q L Q A R L Q V D L E I K
AscI
2341 AGG TGC AGC TGC AGG CGC GCC TGC AGG TCG ACC TCG AGA TCA AAC
TCC ACG TCG ACG TCC GCG CGG ACG TCC AGC TGG AGC TCT AGT TTG
Spacer
Myc-tag
R A A A E Q K L I S E E D L N
NotI
2386 GGG CGG CCG CAG AAC AAA AAC TCA TCT CAG AAG AGG ATC TGA ATG
CCC GCC GGC GTC TTG TTT TTG AGT AGA GTC TTC TCC TAG ACT TAC
Spacer
His tag
G A A H H H H H H * *
EcoRI
2431 GGG CGG CAC ATC ACC ATC ACC ATC ACT AAT AAG AAT TCA CTG GCC
CCC GCC GTG TAG TGG TAG TGG TAG TGA TTA TTC TTA AGT GAC CGG

FIG. 33

PelB-leader

```
+1   M   K   Y   L   L   P   T   A   A   A   G   L   L   L   L
1   ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC
    TAC TTT ATG GAT AAC GGA TGC CGT CGG CGA CCT AAC AAT AAT GAG

                                          VH
+1   A   A   Q   P   A   M   A | E   V   K   L   V   E   S   G
46  GCG GCC CAG CCG GCC ATG GCC|GAG GTG AAG CTG GTG GAG TCT GGG
    CGC CGG GTC GGC CGG TAC CGG|CTC CAC TTC GAC CAC CTC AGA CCC

+1   G   G   L   V   K   P   G   G   S   L   K   L   S   C   A
91  GGA GGC TTA GTG AAG CCT GGA GGG TCC CTG AAA CTC TCC TGT GCA
    CCT CCG AAT CAC TTC GGA CCT CCC AGG GAC TTT GAG AGG ACA CGT

+1   A   S   G   F   T   F   S   S   Y   T   M   S   W   V   R
136 GCC TCT GGA TTC ACT TTC AGT AGC TAT ACC ATG TCT TGG GTT CGC
    CGG AGA CCT AAG TGA AAG TCA TCG ATA TGG TAC AGA ACC CAA GCG

+1   Q   T   P   E   K   R   L   E   W   V   A   T   I   S   S
181 CAG ACT CCG GAG AAG AGG CTG GAG TGG GTC GCA ACC ATT AGT AGT
    GTC TGA GGC CTC TTC TCC GAC CTC ACC CAG CGT TGG TAA TCA TCA

+1   G   G   S   S   T   Y   Y   P   D   S   V   K   G   R   F
226 GGN GGT AGT TCC ACC TAC TAT CCA GAC AGT GTG AAG GGC CGA TTC
    CCN CCA TCA AGG TGG ATG ATA GGT CTG TCA CAC TTC CCG GCT AAG

+1   T   I   S   R   D   N   A   K   N   T   L   Y   L   Q   M
271 ACC ATC TCC AGA GAC AAT GCC AAG AAC ACC CTG TAC CTG CAA ATG
    TGG|TAG AGG TCT|CTG TTA CGG TTC TTG TGG GAC ATG GAC GTT TAC

+1   S | S   L   R | S   E   D   T   A   M   Y   Y   C   T   R
316 AGC|AGT CTG AGG TCT GAG GAC ACA GCC ATG TAT TAC TGT ACA AGA
    TCG TCA GAC TCC AGA CTC CTG TGT CGG TAC ATA ATG ACA TGT TCT

+1   E   G   G   G   F   T   V   N   W   Y   F   D   V   W   G
361 GAG GGG GGT GGT TTC ACC GTC AAC TGG TAC TTC GAT GTC TGG GGC
    CTC CCC CCA CCA AAG TGG CAG TTG ACC ATG AAG CTA CAG ACC CCG

                                                  Leader
+1   A   G   T   S   V   T   V   S   S | G   G   G   G   S   G
406 GCA GGA ACC TCA GTC ACC GTC TCC TCA|GGT GGA GGC GGT TCA GGT
    CGT CCT TGG AGT CAG TGG CAG AGG AGT|CCA CCT CCG CCA AGT CCA
```

FIG. 34-1

```
                                                                VK
  +1  G   R   A   S   G   G   G   G   S | D   I   V   L   T   Q
451  GGG CGC GCC TCT GGC GGT GGC GGA TCG | GAC ATT GTG CTG ACA CAG
     CCC GCG CGG AGA CCG CCA CCG CCT AGC | CTG TAA CAC GAC TGT GTC

  +1  S   P   A   S   L   A   V   S   L   G   Q   R   A   T   I
496  TCT CCA GCT TCT TTG GCT GTG TCT CTA GGG CAG AGG GCC ACC ATA
     AGA GGT CGA AGA AAC CGA CAC AGA GAT CCC GTC TCC CGG TGG TAT

  +1  S   C   R   A   S   E   S   V   D   S   Y   G   Y   N   F
541  TCC TGC AGA GCC AGT GAA AGT GTT GAT AGT TAT GGC TAT AAT TTT
     AGG ACG TCT CGG TCA CTT TCA CAA CTA TCA ATA CCG ATA TTA AAA

  +1  M   H   W   Y   Q   Q   I   P   G   Q   P   P   K   L   L
586  ATG CAC TGG TAT CAG CAG ATA CCA GGA CAG CCA CCC AAA CTC CTC
     TAC GTG ACC ATA GTC GTC TAT GGT CCT GTC GGT GGG TTT GAG GAG

  +1  I   Y   R   A   S   N   L   E   S   G   I   P   A   R   F
631  ATC TAT CGT GCA TCC AAC CTA GAG TCT GGG ATC CCT GCC AGG TTC
     TAG ATA GCA CGT AGG TTG GAT CTC AGA CCC TAG GGA CGG TCC AAG

  +1  S   G   S   G   S   R   T   D   F   T   L   T   I   N   P
676  AGT GGC AGT GGG TCT AGG ACA GAC TTC ACC CTC ACC ATT AAT CCT
     TCA CCG TCA CCC AGA TCC TGT CTG AAG TGG GAG TGG TAA TTA GGA

  +1  V   E   A   D   D   V   A   T   Y   Y   C   Q   Q   S   N
721  GTG GAG GCT GAT GAT GTT GCA ACC TAT TAC TGT CAG CAA AGT AAT
     CAC CTC CGA CTA CTA CAA CGT TGG ATA ATG ACA GTC GTT TCA TTA

  +1  E   D   P   L   T   F   G   T   G   T   R   L   E   I   K
766  GAG GAT CCG CTC ACG TTC GGT ACT GGG ACC AGA CTG GAA ATA AAA
     CTC CTA GGC GAG TGC AAG CCA TGA CCC TGG TCT GAC CTT TAT TTT

          Spacer          Myc-tag
  +1  R | A   A   A | E   Q   K   L   I   S   E   E   D   L   N
811  CGG | GCG GCC GCA | GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG AAT
     GCC | CGC CGG CGT | CTT GTT TTT GAG TAG AGT CTT CTC CTA GAC TTA

          Spacer  His tag
  +1  G | A   A | H   H   H   H   H   H   *   *
856  GGG | GCG GCA | CAT CAC CAT CAC CAT CAC TAA TAA
     CCC | CGC CGT | GTA GTG GTA GTG GTA GTG ATT ATT
```

FIG. 34-2

```
                Pel-leader
      +1  M   K   Y   L   L   P   T   A   A   A   G   L   L   L   L
       1  ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC
          TAC TTT ATG GAT AAC GGA TGC CGT CGG CGA CCT AAC AAT AAT GAG

                                          |VH
      +1  A   A   Q   P   A   M   A   |E   V   Q   L   Q   Q   S   G
      46  GCG GCC CAG CCG GCC ATG GCC |GAG GTT CAG CTT CAG CAG TCT GGA
          CGC CGG GTC GGC CGG TAC CGG |CTC CAA GTC GAA GTC GTC AGA CCT

      +1  P   E   L   V   K   P   G   A   S   V   K   I   S   C   K
      91  CCT GAG CTG GTG AAG CCC GGG GCC TCA GTG AAG ATT TCC TGC AAA
          GGA CTC GAC CAC TTC GGG CCC CGG AGT CAC TTC TAA AGG ACG TTT

      +1  A   S   G   Y   A   F   S   S   S   W   M   N   W   V   K
     136  GCT TCT GGC TAC GCA TTC AGT AGC TCT TGG ATG AAC TGG GTG AAG
          CGA AGA CCG ATG CGT AAG TCA TCG AGA ACC TAC TTG ACC CAC TTC

      +1  Q   R   P   G   Q   G   L   E   W   I   G   R   I   Y   P
     181  CAG AGG CCT GGA CAG GGT CTT GAG TGG ATT GGA CGG ATT TAT CCT
          GTC TCC GGA CCT GTC CCA GAA CTC ACC TAA CCT GCC TAA ATA GGA

      +1  G   N   G   D   T   N   Y   N   G   K   F   K   G   K   A
     226  GGA AAT GGA GAT ACT AAC TAC AAT GGG AAG TTC AAG GGC AAG GCC
          CCT TTA CCT CTA TGA TTG ATG TTA CCC TTC AAG TTC CCG TTC CGG

      +1  T   L   T   A   D   K   S   S   S   T   A   Y   M   Q   L
     271  ACA CTG ACT GCA GAC AAA TCC TCC AGC ACA GCC TAC ATG CAG CTC
          TGT GAC TGA CGT CTG TTT AGG AGG TCG TGT CGG ATG TAC GTC GAG

      +1  S   S   L   T   S   V   D   S   A   V   Y   F   C   A   D
     316  AGC AGC CTG ACC TCT GTG GAC TCT GCG GTC TAT TTC TGT GCA GAT
          TCG TCG GAC TGG AGA CAC CTG AGA CGC CAG ATA AAG ACA CGT CTA

      +1  G   N   V   Y   Y   Y   A   M   D   Y   W   G   Q   G   T
     361  GGT AAC GTA TAT TAC TAT GCT ATG GAC TAC TGG GGT CAA GGA ACC
          CCA TTG CAT ATA ATG ATA CGA TAC CTG ATG ACC CCA GTT CCT TGG

                                      |Leader
      +1  S   V   T   V   S   S   |G   G   G   G   S   G   G   R   A
     406  TCA GTC ACC GTC TCC TCA|GGT GGA GGC GGT TCA GGT GGG CGC GCC
          AGT CAG TGG CAG AGG AGT|CCA CCT CCG CCA AGT CCA CCC GCG CGG

                                  VL
      +1  S   G   G   G   G   S |Q   I   V   L   T   Q   S   P   A
     451  TCT GGC GGT GGC GGA TCG|CAA ATT GTT CTC ACC CAG TCT CCT GCT
          AGA CCG CCA CCG CCT AGC|GTT TAA CAA GAG TGG GTC AGA GGA CGA
```

FIG. 35-1

```
  +1  S   L   A   V   S   L   G   Q   R   A   T   I   S   C   R
496  TCC TTA GCT GTA TCT CTG GGG CAG AGG GCC ACC ATC TCA TGC AGG
     AGG AAT CGA CAT AGA GAC CCC GTC TCC CGG TGG TAG AGT ACG TCC


  +1  A   S   K   S   V   S   T   S   G   Y   S   Y   M   H   W
541  GCC AGC AAA AGT GTC AGT ACA TCT GGC TAT AGT TAT ATG CAC TGG
     CGG TCG TTT TCA CAG TCA TGT AGA CCG ATA TCA ATA TAC GTG ACC

  +1  Y   Q   Q   K   P   G   Q   P   P   K   L   L   I   Y   L
586  TAC CAA CAG AAA CCA GGA CAG CCA CCC AAA CTC CTC ATC TAT CTT
     ATG GTT GTC TTT GGT CCT GTC GGT GGG TTT GAG GAG TAG ATA GAA

  +1  A   S   N   L   E   S   G   V   P   A   R   F   S   G   S
631  GCA TCC AAC CTA GAA TCT GGG GTC CCT GCC AGG TTC AGT GGC AGT
     CGT AGG TTG GAT CTT AGA CCC CAG GGA CGG TCC AAG TCA CCG TCA

  +1  G   S   G   T   D   F   T   L   N   I   H   P   V   E   E
676  GGG TCT GGG ACA GAC TTC ACC CTC AAC ATC CAT CCT GTG GAG GAG
     CCC AGA CCC TGT CTG AAG TGG GAG TTG TAG GTA GGA CAC CTC CTC

  +1  E   D   A   A   T   Y   Y   C   Q   H   S   R   E   L   P
721  GAG GAT GCT GCA ACC TAT TAC TGT CAG CAC AGT AGG GAG CTT CCT
     CTC CTA CGA CGT TGG ATA ATG ACA GTC GTG TCA TCC CTC GAA GGA

                                                        Spacer
  +1  R   T   F   G   G   G   T   K   L   E   I   K   R | A   A
766  CGG ACG TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA CGG | GGG GCC
     GCC TGC AAG CCA CCT CCG TGG TTC GAC CTT TAG TTT GCC | CGC CGG

                Myc-tag                                 Spacer
  +1  A | E   Q   K   L   I   S   E   E   D   L   N   G | A   A |
811  GCA|GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG AAT GGG| GCG GCA|
     CGT|CTT GTT TTT GAG TAG AGT CTT CTC CTA GAC TTA CCC| CGC CGT|

       His tag
  +1  H   H   H   H   H   H   *
856  CAT CAC CAT CAC CAT CAC TAA
     GTA GTG GTA GTG GTA GTG ATT
```

FIG. 35-2

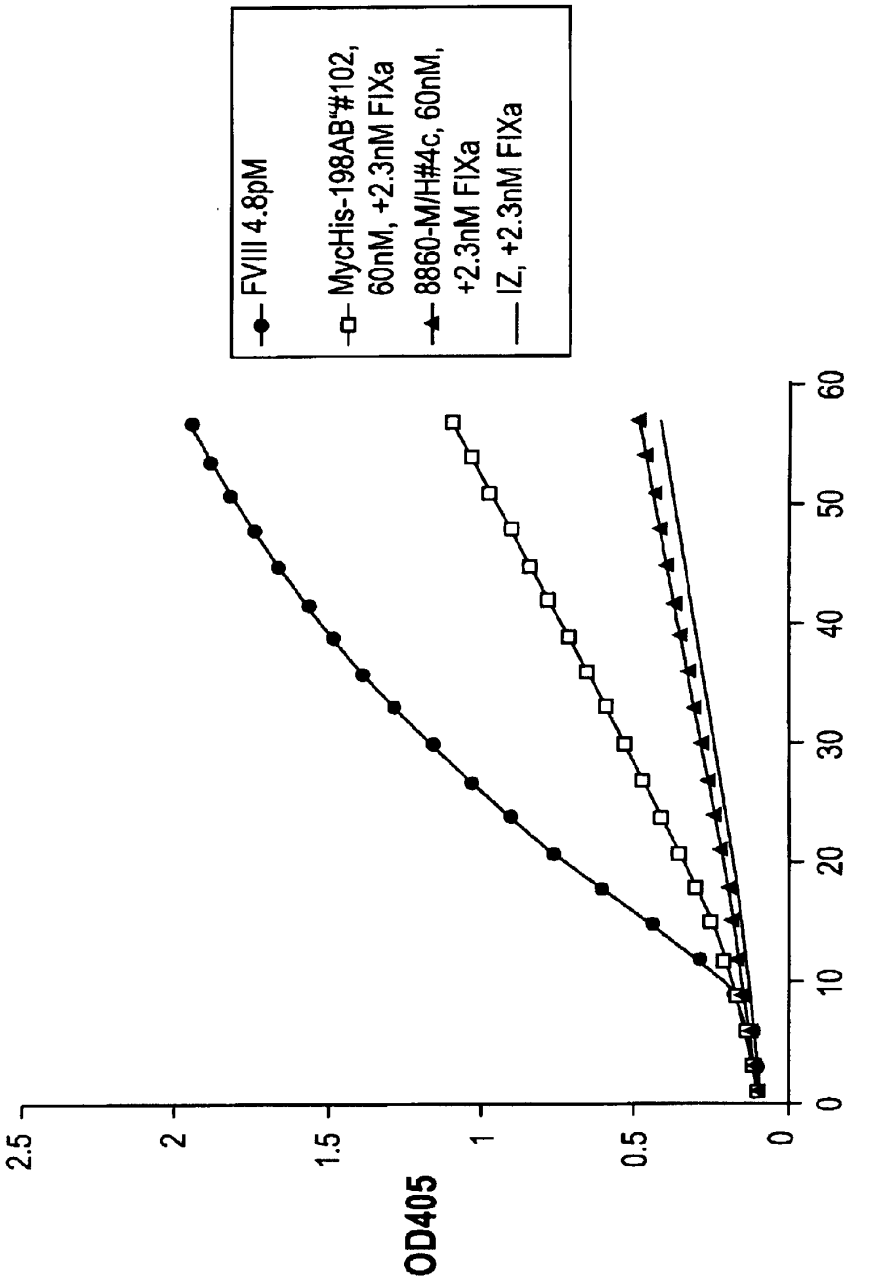

FVIII 4.8pM
MycHis-198AB*#102, 60nM, +2.3nM FIXa
8860-M/H#4c, 60nM, +2.3nM FIXa
IZ, +2.3nM FIXa
OD405
2.5
2
1.5
1
0.5
0
0
10
20
30
40
50
60
time (min)

FIG. 36

FACTOR IX/FACTOR IXA ACTIVATING ANTIBODIES AND ANTIBODY DERIVATIVES

The present invention relates to actor IX/factor IXa-antibodies and antibody derivatives.

Blood clots (thrombi) are formed by a series of zymogen activations referred to as the coagulation cascade. In the course of this enzymatic cascade, the activated form of each of such zymogens (referred to as factors) catalyzes the activation of the next one. Thrombi are deposits of blood components on the surface of a blood vessel wall and mainly consist of aggregated blood platelets and insoluble, cross-linked fibrin. Fibrin formation is effected by means of thrombin by limited proteolysis of fibrinogen. Thrombin is the final product of the coagulation cascade, (K. G. Mann, Blood, 1990, Vol. 76, pp. 1–16).

Activation of factor x by the complex of activated factor IX (FIXa) and activated factor VIII (FVIIIa) is a key step in coagulation. The absence of the components of this complex or a disturbance of their function is associated with the blood coagulation disorder called hemophilia (J. E. Sadler & E. W. Davie: Hemophilia A, Hemophilia B and von Willebrand's disease, in G. Stamatoyannopoulos et al. (Eds.): The molecular basis of blood diseases. W. B. Saunders Co., Philadelphia, 1987, pp. 576–602). Hemophilia A denotes a (functional) absence of factor VIII activity, while Hemophilia B is characterized by the absence of factor IX activity. At present, treatment of Hemophilia A is effected via a substitution therapy by administering factor VIII concentrates. However, approximately 20–30% of Hemophilia A patients develop factor VIII inhibitors (i.e. antibodies against factor VIII), whereby the effect of administered factor VIII preparations is inhibited. Treatment of factor VIII inhibitor patients is very difficult and involves risks, and so far there exist only a limited number of treatments for these patients.

In the case of patients having a low FVIII inhibitor level, it is possible, though expensive, to administer high doses of factor VIII to such patients and thus to neutralize the antibodies against factor VIII. The amount of factor VIII beyond that needed to neutralize the inhibitor antibodies then has hemostatic action. In many cases, desensitization can be effected, whereupon it is then possible again to apply standard factor VIII treatments. Such high dose factor VIII treatments require, however, large amounts of factor VIII, are time-consuming and may involve severe anaphylactic side reactions. Alternatively, the treatment may be carried out with porcine factor VIII molecules.

A further high-cost method involves removing factor VIII inhibitors through extra corporeal immunoadsorption on lectins which bind to immunoglobulins (protein A, protein G) or to immobilized factor VIII. Since the patient must be connected to an apheresis machine during this treatment, the treatment also constitutes a great burden on the patient. It is also not possible to treat an acute hemorrhage in this way.

At present, the therapy of choice is to administer activated prothrombin complex concentrates (APCC), such as FEIBA® and AUTOPLEX®, which are suitable for the treatment of acute hemorrhages even in patients having a high inhibitor titer (DE 31 27 318).

In the intravascular system of blood coagulation, the last step is the activation of factor X. This reaction is stimulated by the binding of factor VIIIa to factor IXa and the formation of a "tenase"-complex consisting of the factors IXa, VIIIa, X and phospholipid. Without the binding of FVIIIa, FIXa exhibits no or only a very slight enzymatic activity relative to FX.

Over the last several years, a number of possible binding sites for factor VIIIa to factor IXa have been characterized, and it has been shown that antibodies or peptides which bind to these regions inhibit the activity of FIXa (Fay et al., J. Biol. Chem., 1994, Vol. 269, pp. 20522–20527, Lenting et al., J. Biol. Chem., 1996, Vol. 271, pp. 1935–1940, Jorquera et al., Circulation, 1992, Vol. 86, Abstract 2725). The inhibition of coagulation factors, such as factor IX, has also been achieved through the use of monoclonal antibodies with the aim of preventing thrombosis formation (WO 97/26010).

The opposite effect, i.e. an increase in the factor IXa mediated activation of factor X, has been described by Liles D. K. et al., (Blood, 1997, Vol. 90, suppl. 1, 2054) through the binding of a factor VIII peptide (amino acids 698–712) to factor IX. Yet, this effect only occurs in the absence of factor VIIIa, while in the presence of factor VIIIa the factor IXa/factor VIIIa-mediated cleavage of factor X is inhibited by this peptide.

SUMMARY OF THE INVENTION

With a view to the possible risks and side effects which may occur in the treatment of hemophilia patients, there is a need for a therapy which allows for the effective treatment of FVIII inhibitor patients. Therefore, it is an object of the present invention to provide a preparation for the treatment of blood coagulation disorders which has particular advantages for factor VIII inhibitor patients.

According to the present invention, this object is achieved through the use of antibodies or antibody derivatives against factor IX/factor IXa which have factor VIIIa-cofactor activity or factor IXa-activating activity and lead to an increase in the procoagulant activity of factor IXa. Surprisingly, the action of these inventive factor IX/factor IXa-activating antibodies and antibody derivatives is not negatively affected by the presence of inhibitors, such as inhibitors against factor VIII/factor VIIIa, but instead the procoagulant activity of factor IXa in this case also is increased.

A further advantage of this invention is that the administration of the preparation according to the invention allows for rapid blood coagulation even in the absence of factor VIII or factor VIIIa, even in the case of FVIII inhibitor patients. Surprisingly, these agents are also effective in the presence of factor VIIIa.

The antibodies and antibody derivatives according to the present invention thus have a FVIII-cofactor-like activity which, in a FVIII assay (e.g. a COATEST® assay or Immunochrom test) after 2 hours of incubation exhibits a ratio of background (basic noise) to measured value of at least 3. Calculation of this ratio may, e.g., be effected according to the following scheme:

$$\frac{\text{Antibody measurement (OD 405)} - \text{blank value from reagent}}{\text{Mouse-IgG-measurement (OD 405)} - \text{blank value from reagent}} \geq 3$$

after two hours of incubation.

The antibodies according to the invention preferably have an in vivo half life of at least 5 days, more preferably at least 10 days, though it is more preferred to have a half life of at least 20 days.

A further aspect of this invention is a preparation comprising antibodies and/or antibody derivatives against factor IX/factor IXa and a pharmaceutically acceptable carrier substance. Furthermore, the preparation according to the invention may additionally comprise factor IX and/or factor IXa.

A further aspect of the invention is the use of the antibodies or antibody derivatives to increase the amidolytic activity of factor IXa.

FIG. **1** shows the results of a screening of supernatants from hybridoma cell cultures for FVIII-like activity. Preselected clones from fusion experiments, #193, #195 and #196, were tested in a chromogenic assay.

FIG. **2** shows the results of screening for IgG-mediated factor VIII-like activity in supernatants of a hybridoma cell culture of a master plate.

FIG. **3** shows the subcloning of clone 193/C0, namely the results of the first cloning round.

FIG. **4** shows a comparison of the chromogenic FVIII-like activity and factor IX-ELISA-reactivity of hybridoma cultures derived from the starting clone 193/C0.

FIG. **5** shows the results of the measurement of the chromogenic activity of some master clones and sub-clones.

FIG. **6**A shows the FVIII-like activity of the anti-FIX/FIXa-antibodies 193/AD3 and 196/AF2 compared to human FVIII, TBS buffer and cell culture medium. After a lag phase, both antibodies gave rise to chromogenic substrate cleavage, as judged by the increasing optical density.

FIG. **6**B shows a comparison of the chromogenic activity of factor VIII, 196/AF1, 198/AC1/1 and mouse-IgG.

FIG. **7**A shows a comparison of the kinetics of Factor Xa generation by Factor VIII and 196/AF2 with and without the addition of a Factor Xa specific inhibitor.

FIG. **7**B shows a comparison of the kinetics of the Factor Xa generation by Factor VIII, mouse-IgG and anti-factor IX/IXa-antibody 198/AM1 with and without the addition of a factor Xa-specific inhibitor, Pefabloc Xa®.

FIG. **8**A shows a measurement of the dependence of the factor VIII-like activity of purified anti-factor IX/IXa-antibody 198/AC1/1 in the presence and absence of phospholipids, FIXa/FX and calcium ions.

FIG. **8**B shows a measurement of the dependence of FXa generation by anti-FIXa-antibody 196/AF1 in the presence of phospholipids, Ca^{2+} in FIXa/FX.

FIG. **8**C shows the generation of FXa by unspecific mouse IgG antibody.

FIG. **9** is a graphical representation of the coagulation times of Factor VIII-deficient plasma in an APTT assay by using various concentrations of anti-factor IX/IXa-antibody 193/AD3.

FIG. **10**A shows that in the presence of Factor IXa, antibody 193/AD3 leads to a reduction in the coagulation time of factor VIII-deficient plasma.

FIG. **10**B shows a dose-dependent reduction of the clotting time by antibody 193/AD3 in the presence of factor IXa- and factor VIII-inhibitors.

FIG. **11** shows the chromogenic activity of antibodies 198/A1, 198/B1 and 198/AP1 in the presence and absence of human FIXaβ.

FIG. **12** shows the primer sequences (SEQ ID NOS:50–61) for the amplification of the genes of the variable heavy chain of mouse antibody.

FIG. **13** shows the primer sequences (SEQ ID NOS:65–78) for the amplification of the genes of the variable light (kappa) chain of the mouse antibody.

FIG. **14** shows the DNA and derived protein sequence of the scFv from hybridoma cell line 193/AD3 (SEQ.ID.NOs. 81 and 82).

FIG. **15** shows the DNA and derived protein sequence of the scFv from hybridoma cell line 193/K2 (SEQ.ID.NOs. 83 and 84).

FIG. **16** shows the DNA and derived protein sequence of the scFv from hybridoma cell line 198/AB2 (subclone of 198/B1) (SEQ.ID.NOs. 85 and 86).

FIG. **17** shows the DNA and deduced protein sequence of scFv derived from the cell line 198/A1 (SEQ.ID.NOs. 87 and 88).

FIG. **18** demonstrates the chromogenic FVIII-like activity of peptide A1/3 in the presence of 2.9 nM human FIXa. The scrambled version of peptide A1/3, peptide A1/5 does not give rise to any FXa generation.

FIG. **19** demonstrates the dependence of the chromogenic FVIII-like activity of peptide A1/3 on the presence of human FIXa. In the absence of human FIXa, peptide A1/3 does not give rise to any FXa generation. The buffer control, plain imidazole buffer is designated IZ.

FIG. **20** shows that the chirality of Arg-residues does not play a significant role for the chromogenic activity of peptides A1/3-rd and A1/3-Rd-srmb.

FIG. **21** shows that the addition of 2.4 μM peptide B1/7 to the reaction mixture led to a measureable generation of FXa.

FIG. **22** shows that the addition of a FX-specific inhibitor results in a significant reduction in the reaction. If there was no FIXa and FX is added to the reaction mixture, no FXa was synthesized.

FIG. **23** shows vector pBax-IgG1.

FIG. **24** shows the increase of the amidolytic activity of FIXa in the presence of antibody 198/B1 (FIG. **24**A) and IgM antibody 198/AF1 (FIG. **24**B).

FIG. **25** demonstrates the chromogenic FVIII-like activity of the antibody 198/A1 Fab fragment in the presence of 2.3 nM human FIXa. As a positive control the intact antibody 198/A1 was used as well as 7.5 pM FVIII. The buffer control (IZ) was used as a negative control.

FIG. **26** shows the nucleotide and amino acid sequence of the 198AB2 scFv-alkaline phosphatase fusion protein (ORF of the expression vector pDAP2-198AB2#100, (SEQ.ID.NOs. 89 and 90).

The genes for the VL and the VH domains of antibody 198/AB2 (198/AB2 is an identical subclone of 198/B1) were derived from the corresponding hybridoma cells as described in example 10. The PCR product of the VH-gene was digested SfiI-AscI and the PCR-product of the VL-gene was digested AscI and NotI. VH and VL genes were linked via the AscI site and inserted into SfiI-NotI digested vector pDAP2 (Kerschbaumer R. J. et al, Immuno-technology 2, 145–150, 1996; GeneBank accession No.:U35316). PelB leader: leader sequence of *Erwinia carotovora* Pectate Lyase B, His tag, Histidinee tag for metal ion chromatography.

FIG. **27** demonstrates the chromogenic FVIII-like activity of two antibody 198/B1 (subclone AB2) scFv fragment-alkaline phosphatase fusion proteins (198AB2#1 and 198AB2#100) in the presence of 2.3 nM human FIXa. As a positive control 7.5 pM FVIII was used.

FIG. **28** shows the amino acid and nucleotide sequence of pZip198AB2#102 (SEQ.ID.NOs. 91 and 92).

FIG. **29** shows the nucleotide and amino acid sequence of the mAB#8860 scFv-alkaline phosphatase fusion protein (vector pDAP2-8860scFv#11, (SEQ.ID.NOs. 93 and 94). The genes for the VT and the VH domains of antibody #8860 were derived from the corresponding hybridoma cells as described in example 10. The PCR product of the VH-gene was digested SfiI-AscI and the PCR-product of the VL-gene was digested AscI and NotI. VH and VL genes were linked via the AscI site and inserted into SfiI-NotI digested vector pDAP2 (Kerschbaumer R. J. et al, Immunotechnology 2, 145–150, 1996; GeneBank accession No.:U35316).

FIG. **30** shows the nucleotide and amino acid sequence of the mAB #8860 scFv-leucine zipper fusion protein

(miniantibody; vector p8860-Zip#1.2, (SEQ.ID.NOs. 95 and 96). The gene of the scFv fragment was derived from mAB #8860 and was swapped from vector pDAP2-8860scFv#11 into SfiI-NotI digested plasmid pZip1 (Kerschbaumer R. J. et al., Analytical Biochemistry 249, 219–227, 1997; GeneBank accession No.: U94951)

FIG. **31** demonstrates the chromogenic FVIII-like activity of the 198/B1 (subclone AB2) miniantibody 198AB-Zip#102 in the presence of 2.3 nM human FIXa. As a positive control 4.5 pM FVIII was used whereas a unrelated miniantibody (8860-Zip#1.2) and plain reaction buffer (IZ) served as negative controls.

FIG. **32** shows a schematic representation of the plasmid pMycHis6 (SEQ ID NOS:107–110).

FIG. **33** shows the nucleotide and amino acid sequence of the part of the plasmid pMycHis6 differing from vector pCOCK (SEQ.ID.Nos. 97 and 98). Vector pMycHis6 was constructed by cleaving vector pCOCK (Engelhardt et al., 1994, Biotechniques, 17: 44–46) with NotI and EcoRI and insertion of the oligonucleotides: mychis6-co: 5'ggccgcagaacaaaaactcatctcagaagaggatct gaatggggcggcacatcaccatcaccatcactaataag 3' (SEQ ID.No. 79) and mycchis-ic: 5' aattcttattagtgatggtgatggtgatgtgccgccccattcagatcctcttctgagatgagtttttgttctgc (SEQ.ID.No. 80).

FIG. **34** shows the nucleotide and amino acid sequence of 198AB2 scFv (linked to the c-myc-tag and the His6tag): ORF of the expression vector pMycHis6-198AB2#102. Vector pMycHis6 was constructed by cleaving vector pCOCK (Engelhardt O. et al, BioTechniques 17, 44–46, 1994) NotI-EcoRI and inserting the following annealed oligonucleotides: (5'-GGCCGCAGAACAAAAACTCATCTCAGAAGAGGATCTGAATGGGGCGGCACATCA CCATCACCATCACTAATAAG-3' (SEQ.ID.No. 103) and 5'-TTATTAGTGATGGTGATGGT GATGTGCC GCCCCATTCAGATCCTCTTCTGAGATGAGTTTTTG TTCTGC-3'(SEQ.ID.NO. 104)). The resultant vector, named pMycHis6, was cleaved SfiI-NotI and the gene of scFv 198AB2 was swapped into this vector from vector pDAP2-198AB2#100.

FIG. **35** shows the nucleotide and amino acid sequence of the mAB #8860 scFv linked to the c-myc-tag and the His6-tag (vector p8860-M/H#4c, SEQ.ID.NOs. 101 and 102). Plasmid pMycHis6 was cleaved with SfiI and NotI and the DNA sequence coding for the scFv 8860#11 protein was inserted from pDAP2-8860scFv#µl (see FIG. **29**) yielding plasmid p8860-M/H#4c.

FIG. **36** demonstrates the chromogenic FVIII-like activity of the 198/B1 (subclone AB2) scFv fragment (MycHis-198AB2#102) in the presence of 2.3 nM human FIXa. As a positive control 4.8 pM FVIII was used whereas a unrelated scFv (8860-M/H#4c) and plain reaction buffer (IZ) served as negative controls.

Antibodies and Antibody Derivatives

The present invention also comprises the nucleic acids encoding the inventive antibodies and antibody derivatives, expression vectors, hybridoma cell lines, and methods for producing the same.

Antibodies are immunoglobulin molecules having a specific amino acid sequence which only bind to antigens that induce their synthesis (or its immunogen, respectively) or to antigens (or immunogens) which are very similar to the former. Each immunoglobulin molecule consists of two types of polypeptide chains. Each molecule consists of large, identical heavy chains (H chains) and two light, also identical chains (L chains). The polypeptides are connected by disulfide bridges and non-covalent bonds. In vivo, the heavy and light chains are formed on different ribosomes, assembled in the cell, and secreted as intact immunoglobulins (Roitt I. et al., in: Immunology, second ed., 1989).

The inventive antibodies and antibody derivatives and organic compounds derived there from comprise human and animal monoclonal antibodies or fragments thereof, single chain antibodies and fragments thereof and miniantibodies, bispecific antibodies, diabodies, triabodies, or di-, oligo- or multimers thereof. Also included are peptidomimetics or peptides derived from the antibodies according to the invention, e.g. they comprise one or several CDR regions, preferably the CDR3 region.

Further included are human monoclonal antibodies and peptide sequences which, based on a structure activity connection, are produced through an artificial modeling process (Greer J. et al., J. Med. Chem., 1994, Vol. 37, pp. 1035–1054).

The term factor IX/IXa activating antibodies and antibody derivatives may also include proteins produced by expression of an altered, immunoglobulin-encoding region in a host cell, e.g. "technically modified antibodies" such as synthetic antibodies, chimeric or humanized antibodies, or mixtures thereof, or antibody fragments which partially or completely lack the constant region, e.g. Fv, Fab, Fab' or $F(ab)'_2$ etc. In these technically modified antibodies, e.g., a part or parts of the light and/or heavy chain may be substituted. Such molecules may, e.g., comprise antibodies consisting of a humanized heavy chain and an unmodified light chain (or chimeric light chain), or vice versa. The terms Fv, Fc, Fd, Fab, Fab' or $F(ab)_2$ are used as described in the prior art (Harlow E. and Lane D., in "Antibodies, A Laboratory Manual", Cold Spring Harbor Laboratory, 1988).

The present invention also comprises the use of Fab fragments or $F(ab)_2$ fragments which are derived from monoclonal antibodies (mAb), which are directed against factor IX/factor IXa and cause an increase of the procoagulant activity of factor IXa.

Preferably, the heterologous framework regions and constant regions are selected from the human immunoglobulin classes and isotypes, such as IgG (subtypes 1 to 4), IgM, IgA and IgE. In the course of the immune response, a class switch of the immuno-globulins may occur, e.g. a switch from IgM to IgG; therein, the constant regions are exchanged, e.g. from p to y. A class switch may also be caused in a directed manner by means of genetic engineering methods ("directed class switch recombination"), as is known from the prior art (Esser C. and Radbruch A., Annu. Rev. Immunol., 1990, Vol. 8, pp. 717–735). However, the antibodies and antibody derivatives according to the present invention need not comprise exclusively human sequences of the immunoglobulin proteins.

In one particular embodiment, a humanized antibody comprises complement determining regions (CDRs) from murine monoclonal antibodies which are inserted in the framework regions of selected human antibody sequences. However, human CDR regions can also be used. Preferably, the variable regions in the human light and heavy chains are technically altered by one or more CDR exchanges. It is also possible to use all six CDRs or varying combinations of less than six CDRs.

The humanized antibody according to the present invention preferably has the structure of a human antibody or of a fragment thereof and comprises the combination of characteristics necessary for a therapeutic application, e.g., the treatment of coagulation disorders in patients, preferably factor VIII inhibitor patients.

A chimeric antibody differs from a humanized antibody in that it comprises the entire variable regions including the framework regions of the heavy and light chains of non-human origin in combination with the constant regions of

both chains from human immuno-globulin. A chimeric antibody consisting of murine and human sequences may, for example, be produced. According to the present invention, the antibodies and antibody derivatives may also be single chain antibodies or miniantibodies (scFv fragments, which, e.g., are linked to proline-rich sequences and oligomerisation domains, e.g. Pluckthun A. and Pack P., Immunotechnology, 1997, Vol. 3, pp. 83–105) or single chain Fv (sFv) which incorporate the entire antibody binding region in one single polypeptide chain. For instance, single chain antibodies may be formed by linking the V-genes to an oligonucleotide which has been constructed as a linker sequence and connects the C terminus of the first V region with the N terminus of the second V region, e.g. in the arrangement VH-Linker-VL or VL-Linker-V_H; both, V_H and V_L thus may represent the N-terminal domain (Huston JS et al., Int. Rev. Immunol., 1993, Vol. 10, pp. 195–217; Raag R. and Whitlow M., FASEB J., 1995, Vol. 9, pp. 73–80). The protein which can be used as linker sequence may, e.g., have a length of up to 150 Å, preferably up to 80 Å, and more preferably up to 40 Å. Linker sequences containing glycine and serine are particularly preferred for their flexibility, or glutamine and lysine, respectively, for their solubility. The choice of the amino acid is effected according to the criteria of immunogenicity and stability, also depending on whether or not these single chain antibodies are to be suitable for physiological or industrial applications (e.g. immunoaffinity chromatography). The single chain antibodies may also be present as aggregates, e.g. as trimers, oligomers or multimers. The linker sequence may, however, also be missing, and the connection of the V_H and V_L chains may occur directly.

Bispecific antibodies are macromolecular, heterobifunctional cross-linkers having two different binding specificities within one single molecule. In this group belong, e.g., bispecific (bs) IgGs, bs IgM-IgAs, bs IgA-dimers, bs $(Fab')_2$, $bs(scFv)_2$, diabodies, and bs bis Fab Fc (Cao Y. and Suresh M. R., Bioconjugate Chem., 1998, Vol. 9, pp. 635–644).

By peptidomimetics, protein components of low molecular weight are understood which imitate the structure of a natural peptide component, or of templates which induce a specific structure formation in an adjacent peptide sequence (Kemp DS, Trends Biotechnol., 1990, pp. 249–255). The peptidomimetics may, e.g., be derived from the CDR3 domains. Methodical mutational analysis of a given peptide sequence, i.e. by alanine or glutamic acid scanning mutational analysis, allows for the identification of peptide residues critical for procoagulant activity. Another possibility to improve the activity of a certain peptide sequence is the use of peptide libraries combined with high throughput screening.

The term antibodies and antibody derivatives may also comprise agents which have been obtained by analysis of data relating to structure-activity relationships. These compounds may also be used as peptidomimetics (Grassy G. et al., Nature Biotechnol., 1998, Vol. 16, pp. 748–752; Greer J. et al., J. Med. Chem., 1994, Vol. 37, pp. 1035–1054).

Examples of hybridoma cells expressing the antibodies or antibody derivatives according to the invention were deposited on 9 Sep. 1999 under the numbers 99090924 (#198/A1), 99090925 (#198/B1) and 99090926 (#198/BB1) and on Dec. 16, 1999 under the numbers 99121614 (#193/A0), 99121615 (#196/c4), 99121616 (#198/D1), 99121617 (198/T2), 99121618 (#198/G2), 99121619 (#198/AC1) and 99121620 (#198/U2) according to the Budapest Treaty.

Methods of Production:

The antibodies of the present invention can be prepared by methods known from the prior art, e.g. by conventional hybridoma techniques, or by means of phage display gene libraries, immunoglobulin chain shuffling or humanizing techniques (Harlow E. and Lane D., in: Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, 1988). The production of the inventive antibodies and antibody derivatives may, for instance, be made by conventional hybridoma techniques (Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, 1988, Eds. Harlow and Lane, pp. 148–242). According to the present invention, human and also non-human species may be employed therefor, such as cattle, pigs, monkeys, chickens and rodents (mice, rats). Normal, immunocompetent Balb/c mice or FIX-deficient mice may, e.g., be used (factor IX-deficient mice may be obtained from Dr. Darrel Stafford from the University of North Carolina, Chapel Hill). Immunization may, e.g., be effected with factor IX, factor IXaα or completely activated factor IXaβ, or with fragments thereof.

The hybridomas are selected with a view to the fact that the antibodies and antibody derivatives in the supernatants of the hybridoma cells bind to factor IX/factor IXa and cause an increase of the procoagulant activity of factor IXa. The increase in the procoagulant activity may, e.g., be proven by assaying methods as known from the prior art for the measurement of factor VIII-like activity, e.g. chromogenic assays.

Alternatively, the antibodies and antibody derivatives of the invention may also be produced by recombinant production methods. In doing so, the DNA sequence of the antibodies according to the invention can be determined by known techniques, and the entire antibody DNA or parts thereof can be expressed in suitable systems. Recombinant production methods can be used, such as those involving phage display, synthetic and natural libraries, expression of the antibody proteins in known expression systems, or expression in transgenic animals (Jones et al., Nature, 19B6, Vol. 321, pp. 522–525; Phage Display of Peptides and Proteins, A Laboratory Manual, 1996, Eds. Kay et al., pp. 127–139; U.S. Pat. No. 4,873,316; Vaughan T. J. et al., Nature Biotechnology, 1998, pp. 535–539; Persic L. et al., Gene, 1997, pp. 9–18; Ames R. S. et al., J.Immunol.Methods, 1995, pp. 177–186).

The expression of recombinantly produced antibodies may be effected by means of conventional expression vectors, such as bacterial vectors, such as pBr322 and its derivatives, pSKF or eukaryotic vectors, such as pMSG and SV40 vectors. Those sequences which encode the antibody may be provided with regulatory sequences which regulate the replication, expression and secretion from the host cell. These regulatory sequences comprise promoters, e.g. CMV or SV40, and signal sequences.

The expression vectors may also comprise selection and amplification markers, such as the dihydrofolate reductase gene (DHFR), hygromycin-B phosphotransferase, thymidine-kinase etc.

The components of the vectors used, such as selection markers, replicons, enhancers etc., may either be commercially obtained or prepared by means of conventional methods. The vectors may be constructed for the expression in various cell cultures, e.g. for mammalian cells such as CHO, COS, fibroblasts, insect cells, yeast or bacteria, such as *E. coli*. Preferably, those cells are used which allow for an optimal glycosylation of the expressed protein. Particularly preferred is the vector pBax (cf. FIG. **17**) which is expressed in CHO cells or in SK-Hep.

The production of Fab fragments or $F(ab)_2$ fragments may be effected according to methods known from the prior art, e.g. by cleaving a mAb with proteolytic enzymes, such as

papain and/or pepsin, or by recombinant methods. These Fab and $F(ab)_2$ fragments may also be prepared by means of a phage display gene library (Winter et al., 1994, Ann. Rev. Immunol., 12: 433–455).

The antibody derivatives may also be prepared by means of methods known from the prior art, e.g. by molecular modeling, e.g. from Grassy G. et al., Nature Biotechnol., 1998, Vol. 16, pp. 748–752, or Greer J. et al., J. Med. Chem., Vol. 37, pp. 1035–1054, or Rees A. et al., in: "Protein Structure Prediction: A practical approach", ed. Sternberg M. J. E., IRL press, 1996, chapt. 7–10, pp. 141–261.

The purification of the inventive antibodies and antibody derivatives may also be carried out by methods described in the prior art, e.g., by ammonium sulfate precipitation, affinity purification (protein G-Sepharose), ion exchange chromatography, or gel chromatography. The following methods may be used as the test methods to show that the antibodies and antibody derivatives of the present invention bind to factor IX/factor IXa, increase the procoagulant activity of factor IXa or have factor VIII-like activity.: the one step coagulation test (Mikaelsson and Oswaldson, Scand. J. Haematol., Suppl., 33, pp. 79–86, 1984) or the chromogenic tests, such as COATEST VIII:C® (Chromogenix) or Immunochrom (IMMUNO). In principle, all the methods used for determining factor VIII activity may be used. As the control blank value for the measurements, e.g., unspecific mouse-IgG antibody may be used.

The present antibodies and antibody derivatives are suitable for therapeutic use in the treatment of coagulation disorders, e.g. in the case of hemophilia A, for factor VIII inhibitor patients etc. Administration may be effected by any method suitable to effectively administer the therapeutic agent to the patient, e.g. by oral, subcutaneous, intramuscular, intravenous or intranasal administration.

Therapeutic agents according to the invention may be produced as preparations which comprise a sufficient amount of antibodies or of antibody derivatives as the active agent in a pharmaceutically acceptable carrier substance. These agents may be present either in liquid or in powderized form. Moreover, the preparations according to the invention may also comprise mixtures of different antibodies, the derivatives thereof and/or organic compounds derived therefrom, as well as mixtures consisting of antibodies and factor 1× and/or factor IXa. Factor IXa may be present as factor IXaα and/or factor IXaβ. An example of an aqueous carrier substance is, e.g., saline. The solutions are sterile, sterilisation being effected by conventional methods.

The antibodies or antibody derivatives according to the invention may be present in lyophilized form for storage and be suspended in a suitable solvent before administration. This method has proven generally advantageous for conventional immunoglobulins, and known lyophilisation and reconstitution methods may be applied in this case.

Moreover, the antibodies and antibody derivatives according to the invention may also be used for industrial applications, e.g. for the purification of factor IX/factor IXa by means of affinity chromatography, or as a component of detection methods (e.g. ELISA assays), or as an agent for identification of and interaction with functional domains of a target protein.

The present invention will be described in more detail by way of the following examples and drawing figures, to which, however, it shall not be restricted.

EXAMPLES

Example 1

Immunization of Immunocompetent Mice and Generation of Anti-FIX/IXa Antibody Secreting Hybridoma Cells

Groups of 1–3 normal immunocompetent 5–8 week old Balb/c mice were immunized with 100 μg antigen (100 μl doses) via the intraperitoneal (i.p.) route. In a typical experiment, mice were inoculated with either recombinant human coagulation factor (F) IX (Benefix™), human activated FIXaα (Enzyme Research Laboratories, Lot: FIXaα 1190L) or human FIXaβ (Enzyme Research Laboratories, Lot: HFIXAaβ 1332 AL,) adjuvanted with $Al(OH)_3$ or KFA.

Individual mice were boosted at various times with 100 μg antigen (10011 doses, i.p) and sacrificed two days later. Spleen cells were removed and fused to P3×63-Ag8 6.5.3 myeloma cells essentially as described by Lane et al., 1985 (J. Immunol. Methods, Vol. 81, pp. 223–228). Each fusion experiment was individually numbered, i.e. #193, 195, 196 or 198.

Hybridoma cells were grown in 96 well plates on a macrophage feeder layer (app. 10^5 cells/ml) and selected in HAT-medium (RPMI-1640 medium supplemented with antibiotics, 10% FCS, Na-pyruvate, L-glutamine, 2-mercaptoethanol and HAT (HAT 100x: 1.0×10^{-2}M hypoxanthine in H_2O (136.1 mg/100 ml H_2O), 4.0×10^{-5}M aminopterin in H_2O (1.76 mg/100 ml H_2O) and 1.6×10^{-3}M thymidine in H_2O (38.7 mg/100 ml H_2O). Medium was first changed after 6 days and thereafter twice a week. After 2–3 weeks HAT-medium was changed to HT-medium (RpMI-1640 supplemented with antibiotics, 10% FCS, Na-pyruvate, L-glutamine, 2-mercaptoethanol and HT) and later on (after additional 1–2 weeks) to normal growth medium (RPMI-1640 medium supplemented with 10% FCS, Na-pyruvate, L-glutamine and 2-mercaptoethanol) (see: HYBRIDOMA TECHNIQUES, EMBO, SKMB Course 1980, Base1).

In another set of experiments FIX deficient C57B16 mice (Lin et al., 1997, Blood, 90: 3962) were used for immunization and subsequent hybridoma production. Since FIX knockout (k.o.) mice do not express endogenous FIX, the anti (a)-FIX antibody spectrum achievable is supposed to be different compared to normal Balb/c mice (due to lack of tolerance).

Example 2

Assaying for FVIII-like Activity in Supernatants of Anti-Fix/FIXa Antibody Secreting Hybridoma Cells

In order to assay the FVIII-like activity of anti-FIXa antibodies secreted by hybridoma cells, the commercially available test-kit COATEST VIII:C/4® (Chromogenix) was employed. The assay was done essentially as described by the manufacturer with the following modifications:

To allow high throughput screening, the assay was downscaled to microtiter plate format. Briefly, 25 μl aliquots of hybridoma supernatants were transferred to microtiter plate (Costar, #3598) wells and warmed to 37° C. Chromogenic substrate (S-2222), synthetic thrombin inhibitor (1–2581), factor (F) IXa and FX were reconstituted in sterile water and FIXa/FX was mixed with phospholipids according to the supplier's protocol. Per reaction, 50 μl of the phospholipid/FIXa/FX solution were combined with 25 μl $CaCl_2$ (25 mM) and 50 μl of the substrate/inhibitor cocktail. To start the reaction, 125 μl of the premix were added to the hybridoma supernatant in the microtiter plates and incubated at 37° C. Absorbency at 405 nm and 490 nm of the samples was read at various times (30 min to 12 h) against a reagent blank (MLW, cell culture medium instead of hybridoma supernatant) in a Labsystems iEMS Reader MF™ microtiter plate reader. FVIII-like activity of the samples was calculated by comparing the absorbency of the samples against the absorbency of a diluted FVIII reference standard (IMMUNO AG # 5T4AR00) using GENESIS™ software.

The results of a screening for FVIII-like activity in hybridoma cell culture supernatants are shown in FIG. **1**. Pre-selected clones derived from fusion experiments #193, #195 and #196 (see above) were examined in a chromogenic FVIII assay as described. Clones 193/M1, 193/N1 and 193/P1 are subclones derived from the master clone 193/C0 (see below). Master clone 195/10 was derived from fusion experiment #195 and clones 196/A0, 196/B0 and 196/C0 were derived from fusion experiment #196. In a typical screening experiment, approximately 1000 clones (in 96 wells) from a single fusion experiment were pre-screened for FVIII-like activity. Subsequently, selected clones were grown on a larger scale (3–5 ml supernatant) and re-analyzed in a chromogenic assay. As a negative control cell culture medium was assayed on each plate (MLW).

Wells either exhibiting high FVIII-like activity or substantial FVIII-like activity were subjected to subcloning procedures. The selection and subcloning process is exemplified for the screening and subcloning of an IgG producing cell line (i.e. 193/C0) but has been done exactly the same way for an IgM (i.e. 196/C0, see below, FIG. **5**) producing clone.

The selection process was done by initially plating all hybridoma cell clones derived from a single fusion experiment on ten 96 well plates thereby creating the so called "master plates". Singular positions (wells) on a master plate usually contained more than one hybridoma cell clone (usually 3 to 15 different clones). Subsequently, the antibody secreted by only several thousand cells was tested. These cells grew under conditions suboptimal for antibody production, which is known to be best in dying cells. So the expected specific anti-FIX antibody concentration in the supernatant may be in the range of 10–12 to 10–14 M. This explains why incubation periods had to be extended compared to standard FVIII assays.

Results of a screening for an IgG mediated FVIII-like activity in hybridoma cell culture supernatants of a master plate are shown in FIG. **2**. Supernatants were examined in a chromogenic FVIII assay. Shown are the results derived from the fifth master plate of fusion experiment number #193 (Balb/c mice immunized with FIXaα). Absorbance was read after 4 hours of incubation at 37° C. Position ES was identified as exhibiting FVIII like activity significantly higher than the blank (MLW). This cell pool was designated 193/C0 and was further subcloned (FIG. **3**). As each well of the master plate contains more than one hybridoma cell clone, cells of a single positive well were expanded and plated at a calculated cell density of 2–0.2 cells/well on a 96 well plate. Again, the supernatants were tested for FVIII-like activity and positive positions were subjected to another round of subcloning. Typically three to four rounds of subcloning were performed with each clone displaying FVIII-like activity to obtain homogenous cell populations. Here the results of the chromogenic assay of the 193/C0 subclones are shown. Absorbance was read after a 4 hour incubation period at 37° C. Positions A6 and D5 exhibited substantial FVIII-like activity and were named 193/M1 and 193/P1, respectively. These two clones were subjected to another round of subcloning. As a negative control plain cell culture medium was assayed on each plate (MLW(H1)).

A comparison of chromogenic FVIII-like activity and FIX-ELISA reactivity of small scale (3 ml) hybridoma cultures is shown in FIG. **4**. Before a decision was made whether a master clone (or subclone) was to be further subcloned, clones were grown at a 3–5 ml scale and the supernatants were checked again. This graph shows the FIX specific ELISA results and the FVIII-like chromogenic activity of the master clone 193/C0 and all its subclones which were identified as positives and re-checked. Blanks (absorbency of the chromogenic reagent itself) were subtracted in the case of the ELISA as well as the chromogenic assay readings depicted here. Clone 193/M1 was subcloned and yielded clones 193/V2, 193/M2 and 193/U2. The other clones of the 2^{nd} round came from 193/P1, 193/AB2 and 193/P2 were subcloned. 193/AF3, 193/AB3 and 193/AE3 are subclones of 193/AB2. The other clones of the 3^{rd} round came from 193/P2. Finally 193/AF3 (→>193/AF4), AE3 (→193/AE4, 193/AL4, 193/AN4 and 193/AO4) and 193/AD3 (→193/AG4, 193/AH4, 193/AD4, 193/AI4, 193/AK4) were subcloned.

From each fusion experiment, several (5–15) master clones (selected from the master plate) were identified and subjected to subcloning. After 3 rounds of sub-cloning, most of the cell lines were homogenous as demonstrated by ELISA and chromogenic activity analysis (see FIG. **4**) as well as by cDNA sequence analysis. A specific master clone and all its subclones produce the same FIX/FIXa binding antibody. However, there are huge differences in the antibody protein sequences of clones derived from different master clones (see Example 11). Most hybridoma cell lines express antibodies from the IgG subclass (i.e. clones #193, #198, like 198/A1, 198/B1, 198/BB1). However, we were also able to select some clones expressing IgM antibodies.

The chromogenic activity of hybridoma supernatant of some important master clones and subclones was determined. Absorbance was measured after a 1 h 30 min and 3 h 30 min incubation period at 37° C. (FIG. **5**). In contrast to all the clones from the 193^{rd} fusion, clone 196/C0 and its subclone 196/AP2 produced a FIX/FIXa-specific IgM antibody that gave a strong chromogenic activity even after a short period of incubation.

The following cell lines have been deposited with the European Collection of Cell Cultures (ECACC) in accordance with the Budapest Treaty: 198/B1 (ECACC No. 99090925, deposited Sep. 9, 1999); 198/A1 (ECACC No. 99090924, deposited Sep. 9, 1999); 198/BB1 (ECACC No. 99090926, deposited Dec. 16, 1999); 193/AO (ECACC No. 99121614, deposited Dec. 16, 1999); 196/C4 (ECACC No. 99121615, deposited Dec. 16, 1999); 198/DI (ECACC No. 99121616, deposited Dec. 16, 1999); 198/T2 (ECACC No. 99121617, deposited Dec. 16, 1999); 198/G2 (ECACC No. 99121618, deposited Dec. 16, 1999); 198/AC1 (ECACC No, 99121619, deposited Dec. 16, 1999); and 198/U2 (ECACC No. 99121620, deposited Dec. 16, 1999). The address of the ECACC is Salisbury. Wiltshire SP4 OJG, UK.

To do a more in depth analysis of the biochemical properties of certain antibodies, homogenous hybridoma cell lines expressing different antibodies with FVIII-like activity were expanded and used to express the antibody in question on a larger scale (100–1000 ml). These antibodies were affinity purified (see Example 3) prior to being used in further experiments.

Example 3

Factor IX/FIXa$_{(\alpha,\beta)}$ Binding Properties of Antibodies Exhibiting FIX/FIXa Activating Activity

Factor IX and the two activated forms of FIX, FIXaα and FIXaβ (FIX/FIXa$_{(\alpha,\beta)}$) were diluted in TBS (25 mM Tris HCl, 150 mM NaCl, pH 7.5) to a final concentration of 2 μg/ml. Nunc Maxisorp ELISA plates were coated with 100 μl FIX/FIXa$_{(\alpha,\beta)}$ solution according to standard procedures (4° C., overnight) and washed several times with TBST

(TBS, 0.1% (v/v) Tween 20). 50 μl hybridoma supernatant was diluted 1:1 with 50 μl TBST/2% BSA and added to the coated ELISA plate. After an incubation period of 2 h at room temperature (RT), plates were washed 4 times with TBST and incubated (2 h, RT) with 100 μl/well of a 1:25000 dilution (in TBST/1% BSA) of an anti-mouse IgG (Fc-specific) peroxidase conjugated antibody (Sigma, #A-0168). Wells were washed 5 times with TBST and finally stained with 100 μl freshly prepared staining solution (10 ml 50M sodium citrate, pH 5 supplemented with 100 μl OPD (60 mg OPD/ml) and 10 μl 30% H_2O_2). The reaction was stopped by the addition of 50 ml H_2S_4 and the optical density recorded at 492 nm and 620 nm in a Labsystems iEMS Reader MF™ microtiter plate reader employing GENESIS™ software.

In certain cases, instead of an anti-mouse IgG ELISA, an anti-mouse IgM ELISA was carried out.

Purification of Mouse-IgG from Hybridoma Cell Culture Supernatants

Hybridoma supernatant (100–500 ml) was supplemented with 200 mM Tris/HCl buffer (pH 7.0) and solid NaCl to give final concentrations of 20 mM Tris and 3M NaCl, respectively. The supernatant was then clarified by centrifugation at 5500×g for 10 minutes. A 1 ml protein G affinity chromatography column (Protein G Sepharose Fast Flow, Amersham-Pharmacia) was washed with 15 ml 20 mM Tris/Cl pH 7.0 and afterwards equilibrated with 10 ml of 20 mM Tris/Cl buffer pH 7.0 containing 3M NaCl. The hybridoma supernatant containing 3M NaCl was then loaded onto the column by gravity. The column was washed with 15 ml of 20 mM Tris/Cl buffer, pH 7.0, containing 3M NaCl. Bound IgG was further eluted with 12 ml glycine/HCl buffer pH 2.8 and 1 ml fractions were collected. 100 μl of 1M Tris pH 9.0 were added to each fraction for neutralization. Fractions containing the IgG were identified by mixing 50>1 with 150 μl of a staining solution (BioRad concentrate, 1:5 diluted with water) in wells of a microplate. Positive fractions were pooled, concentrated to 1 ml in an ultrafiltration concentrator device (Centricon Plus 20, Amicon) according to the manufacturer. The concentrate was diluted with 19 ml TBS (20 mM Tris/Cl buffer pH 7.0 containing 150 mM NaCl) and again concentrated to 1 ml. The diluting-concentrating step was repeated for two more times in order to bring IgG into TBS.

Purification of Mouse-IgM from Hybridoma Cell Supernatants

100–500 ml of hybridoma cell culture supernatant were concentrated to 5–10 ml either with an ultra-filtration concentrator device (Centricon Plus 20, Amicon) according to the manufacturer or by ammonium sulfate precipitation (40% saturation, 0° C.) and redissolving the precipitate with 5–10 ml of TBS. In either case the concentrate was dialyzed against 20 mM Tris Cl buffer pH 7.4 containing 1.25M NaCl and further concentrated to 1 ml in a Centricon Plus 20, (Amicon) ultrafiltration device. IgM was purified from this concentrate with the Immunopure IgM Purification Kit (Pierce) according to the manufacturer. Fractions collected during elution from the maltose binding protein-column were tested for IgM, pooled, concentrated and brought into TBS as described for IgG.

Determination of IgG Concentrations in Purified Preparations

Total IgG content 280 nm−extinction of appropriate dilutions were measured. E280=1.4 corresponds to 1 mg/ml protein.

Factor IXa Specific IgG (Quantitative ELISA)

Wells of a microplate (Nunc Maxisorp) were incubated with 2 μg/ml factor IXa diluted in TBS (25 mM Tris/HCl pH 7.5 containing 150 mM NaCl) overnight at 4° C. Wells were washed four times with TBST (25 mM Tris/HCl pH 7.5 containing 150 mM NaCl and 0.1% (v/v) Tween 20). As a standard monoclonal AB the HIX1 anti-FIX (accurate) was used. Standard and samples were diluted in TBST containing 2%(w/v) BSA. The standard dilution series and appropriate dilutions of the samples were incubated on the ELISA-plate for 2 hours at room temperature. Plates were washed 4 times with TBST and incubated (2 h, RT) with 100 μl/well of a 1:25000 dilution (in TBST/1% BSA) of an anti-mouse IgG (Fc-specific) peroxidase conjugated antibody (Sigma, #A-0168) FIXa. Wells were washed 5 times with TBST and finally stained with 100 μl freshly prepared staining solution (10 ml 50 mM sodium citrate, pH 5 supplemented with 100 μl OPD (60 mg OPD/ml) and 10 μl 30% H_2O_2). The reaction was stopped by the addition of 50 ml H_2SO_4 and after 30 minutes the optical density was recorded at 492 nm and 620 nm in a Labsystems iEMS Reader MF™ microtiter plate reader employing GENESIS™ software.

Example 4

Anti-FIX/FIXa Antibodies Exhibiting FVIII-like Activity in a Chromogenic FVIII Assay

Several anti-FIX/FIXa antibody producing hybridoma clones were subcloned up to four times and the resulting monoclonal hybridoma cell line used to produce monoclonal antibody containing supernatant. IgG isotype antibodies derived from these supernatants were purified over affinity columns and dialyzed against TBS (see above). IgM antibodies were used as unpurified supernatant fractions. The following experiments were done with two sets of representative antibodies: 193/AD3 and 198/AC1/l (IgG isotype, the antibody 198/AC1/1 is a preparation from the parent 198/AC1 hybridoma clone, i.e. that a (frozen) vial containing 198/AC1 cells is cultivated and antibodies are produced. The supernatant is then used for these experiments.) and 196/AF2 and 196/AF1 (IgM isotype) (FIG. **6**A and FIG. **6**B). Briefly, 25 μl aliquots of monoclonal antibody containing sample (unpurified hybridoma supernatant or, where indicated, a certain amount of FIX specific antibody) were transferred to microtiter plate wells and warmed to 37° C. Chromogenic substrate (S-2222), synthetic thrombin inhibitor (I-2581), factor (F) IXa and FX were reconstituted in sterile water and FIXa/FX was mixed with phospholipids according to the supplier's protocol. Per reaction, 50 μl of the phospholipid/FIXa/FX solution were combined with 25 μl $CaCl_2$ (25 mM) and 50 μl of the substrate/inhibitor cocktail. To start the reaction, 125 μl of the premix were added to the monoclonal antibody solution in the microtiter plates and incubated at 37° C. Absorbance at 405 nm and 490 nm of the samples was read at various times (5 min to 6 h) against a reagent blank (cell culture medium instead of hybridoma supernatant) in a Labsystems iEMS Reader MF™ microtiter plate reader using GENESIS™ software.

The time course of FVIII-like activity exhibited by monoclonal antibodies 193/AD3 (IgG isotype) and 196/AF2 (IgM isotype) compared to human FVIII (12 and 16 mU/ml), TBS and to cell culture medium is shown in FIG. **6**A. After a lag phase, both antibodies give rise to chromogenic substrate cleavage, as judged by the increasing optical density measurable at 405 nm wavelength.

The time course of FVIII-like activity exhibited by monoclonal antibodies 198/AC1/1 (IgG isotype, 10 μg/ml) and 196/AF1 (IgM isotype, unpurified supernatant) compared to human FVIII (16 mU/ml) and 10 μg/ml of mouse IgG is

shown in FIG. **6**B. After a lag phase, both antibodies give rise to chromogenic substrate cleavage, as judged by the increasing optical density measurable at 405 nm wavelength.

Example 5

FVIII-like Activity Exhibited by Anti-FIX/FIXa-antibodies Generates Factor Xa and is Phospholipid, FIXa/FX and Ca^{2+} Dependent

Factor VIII activity is usually determined with a chromogenic assay and/or an APTT-based clotting assay. Both types of assays rely on FVIIIa/FIXa-mediated factor Xa generation. In the case of a chromogenic FVIII assay, the factor Xa produced will subsequently react with a chromogenic substrate, which can be monitored spectroscopically, e.g., in an ELISA reader. In an APTT based clotting assay free factor Xa will assemble with FVa on a phospholipid surface in the so-called prothrombinase complex and activate prothrombin to thrombin. Thrombin in turn gives rise to fibrin generation and finally to clot formation. Central to the two assay systems is generation of factor Xa by the FVIIIa/FIXa complex.

To demonstrate that the FVIII-like activity exhibited by anti-FIX/FIXa-antibodies indeed generates factor Xa, the following experiment was carried out. Several 25 μl aliquots of unpurified hybridoma supernatant 196/AF2 (IgM isotype) were transferred to microtiter plate wells and warmed to 37° C. As a positive control, 16mU of Recombinate™ were diluted into hybridoma medium (196 HM 007/99) and treated exactly the same way as the hybridoma supernatant. As a negative control, plain hybridoma medium was used. Chromogenic substrate (S-2222), synthetic thrombin inhibitor (I-2581), factor IXa and FX were reconstituted in sterile water and FIXa/FX was mixed with phospholipids according to the supplier's protocol. Pefabloc Xa®, a factor Xa specific proteinase inhibitor (Pentapharm, LTD), was reconstituted with water to a final concentration of 1 mM/l. Per reaction, 5011 of the phospholipid/FIXa/FX solution were combined with 25 μl $CaCl_2$ (25 mM) and 50 μl of the substrate/thrombin-inhibitor cocktail. To start the reaction, 125 μl of the premix were added to the samples in the microtiter plates and incubated at 37° C. Where indicated, 35 μM Pefabloc Xa® were added. Absorbance at 405 nm and 490 nm was read at various times (every 5 minutes to 6 h) against a reagent blank (cell culture medium) in a Labsystems iEMS Reader MF™ microtiter plate reader employing the GENESIS™ software.

The results of the factor IXa stimulation by the FVIII-like activity exhibited by the IgM anti FIX/FIXa-antibody 196/AF2 in generating actor Xa as judged by the readily measurable cleavage of the chromogenic substrate S-2222 (compare "16 mU FVIII" and "196/AF2") is shown in FIG. **7**A. Factor Xa activity is effectively blocked by the FXa specific inhibitor "Pefabloc Xa®" (compare "196/AF2" versus "196/AF2 351M Pefabloc Xa®") indicating that indeed FXa was generated.

The same experiment was performed using purified IgG preparations of clone 198/AM1 (FIG. **7**B). Purified IgG was diluted in TBS to a final concentration of 0,4 mg/ml and 25 μl (i.e. a total of 10 μg), transferred to microtiter plate wells and warmed to 37° C. As a positive control, 6 mU plasma derived FVIII was used. 10 μg unspecific mouse IgG (Sigma, I-5381) served as a negative control. The assay was performed as described above.

Further experiments show the factor IXa stimulation by the FVIII-like activity exhibited by the IgG anti-FIX/FIXa-antibody 198/AM1 generates factor Xa as judged by the readily measurable cleavage of the chromogenic substrate S-2222 (FIG. **7**B). Again factor VIII and antibody 198/AM1 generate FXa which is effectively blocked by the FXa specific inhibitor "Pefabloc Xa®". As a negative control, unspecific mouse IgG (Sigma, 15381) was assayed.

In another set of experiments, the dependence of the FVIII-like activity of either purified anti-FIX/FIXa-antibodies (IgM, FIG. **8**A) or of unpurified antibodies derived from cell culture supernatants (IgG, FIG. **8**B) on the presence of phospholipids (PL), FIXa/FX and Ca^{2+} was demonstrated. Mouse IgG was used as a control (FIG. **8**C). Factor VIII-like activity was assayed essentially as described above. When indicated, either the FIXa/FX mixture, the PL or Ca^{2+} was omitted from the reaction. Absorbency at 405 nm and 490 nm of the samples was read at various times against a reagent blank (buffer instead of purified antibody) in a Labsystems iEMS Reader MF™ microtiter plate reader. The results are shown in FIG. **8**A, FIG. **8**B and FIG. **8**C.

The dependence of the FVIII-like activity of purified anti-FIXa-antibody 198/AC1/1 (IgG isotype, concentration used throughout the assay was 10 μg/ml) on the presence of phospholipids (PL), FIXa/FX and Ca^{2+} is further shown in FIG. **8**A. As is easily recognizable, only the complete assay, including antibody, PL, Ca^{2+}, and FIXa/FX gives rise to a reasonable FXa generation. The dependence of the FVIII-like activity of cell culture supernatant containing unpurified IgM isotype anti-FIX/FIXa-antibody (196/AF1) on the presence of phospholipids, FIXa/FX and Ca^{2+} is shown in FIG. **8**B.

Again, as already shown for the purified IgG preparation (FIG. **8**A), antibody 198/AC1/1, only the complete assay, including PL, Ca^{2+}, FIXa/FX, will give a reasonable amount of FXa generation. To demonstrate the specificity of the reaction, total IgG prepared from normal mouse plasma was assayed under the same conditions as above. The results are shown in FIG. **8**C. No FVIII-like activity could be detected. There is, as expected, no activity detectable in the absence of phospholipids, FIXa/FX and Ca^{2+}. All experiments were done in a microtiter plate and the OD405 was scanned every 5 minutes for 6 h.

Example 6

Certain anti-FIX/FIXa-antibodies are procoagulant in the presence of FIXa

During normal hemostasis, FIX becomes initially activated either by the tissue factor (TF)/factor VIIa pathway or later on by activated factor XI (FXIa). Subsequent to its activation, FIXa associates on the platelet surface in a membrane bound complex with activated FVIII. Factor IXa by itself has little or no enzymatic activity towards FX, but becomes highly active in the presence of FVIIIa. To demonstrate that certain anti-FIX/FIXa antibodies have FVIII-like activity and hence are procoagulant in a FVIII deficient human plasma, the following experiment was carried out. Different amounts of antibody 193/AD3 or mouse IgG (as a control) were used in a standard aPTT based one stage clotting assay. Briefly, 100 μl of antibody-containing samples were incubated with 100 μl of FVIII deficient plasma (DP) and with 100 μl of DAPTTIN (PTT Reagent for determining activated Thromboplastin Time; IMMUNO AG) reagent, in a KC10A clotting analyzer. Where indicated, a total amount of 50 ng activated FIX was included in the reaction mixture. After a 4 minute

incubation, the reaction was started by the addition of 100 µl $CaCl_2$ (25 mM). The results are shown in Table 1 and FIG. **9**.

	clotting time (sec)	
µg AB	193/AD3 50 ng FIXa	mouse IgG 50 ngFIXa
9	101.6	102.5
4.5	95.6	103.2
2.25	93.1	103.2
1.8	93.7	101.9
1.35	91.4	103.4
0.9	94.4	102.2
0.45	98.1	101.9
0.34	97.1	103.9
0.23	99.3	103.7

Table 1: Clotting times of FVIII deficient plasma in an APTT based clotting assay employing various amounts of procoagulant (193/AD3) and control antibody (mouse IgG) in the presence of 50 ng activated FIX (0.01UFIX). The molar ratio of antibody in the reaction and activated FIX is 10:1. The molar ratio between antibody and total FIX (FIX and FIXa, assuming that human FVIII deficient plasma contains 1 U (5% g) FIX) varies between 6:1 (9% g antibody in reaction) and 1:6 (0.23% g antibody in reaction). At the optimal shortening of the clotting time, the molar ratio between antibody and total FIX is 1:1. The clotting time without the addition of FIXa is in the range of 120 seconds.

FIG. **9** is a graphical representation of the clotting times of FVIII deficient plasma in an aPTT based clotting assay employing various amounts of procoagulant (193/AD3) and control (mouse IgG) antibody in the presence of 50 ng activated FIX. There is a clear dose-dependent reduction of the clotting time in samples supplemented with antibody 193/AD3. These results imply that antibody 193/AD3 is procoagulant in the presence of FIXa.

Example 7

Anti-FIX/FIXa-antibodies are Procoagulant in the Presence of FVIII Inhibitors and FIXa

A severe complication of the standard FVIII substitution therapy is the development of alloantibodies directed against FVIII, leading to FVIII neutralization and a condition where the patient's blood will not clot.

To demonstrate that certain anti-FIXa-antibodies have FVIII-like activity even in the presence of FVIII inhibitors, the following experiment was carried out. Different amounts of antibody 193/AD3 or, as a control, mouse IgG were used in a standard APTT based one-stage clotting assay. Briefly, 100 µl antibody samples were incubated with either 100 µl of FVIII deficient plasma (FIG. **10**A) or FVIII inhibitor plasma (inhibitor potency 400 BU/ml), FIG. **10**B) as well as with 100 µl of DAPTTIN reagent, in a KC10A clotting analyzer. In addition, a total amount of 50 ng activated FIXa was included in the reaction mixture. After a 4 minute incubation, the reaction was started by the addition of 100 µl $CaCl_2$ (25 mM). To ensure equal conditions, the experiments employing FVIII deficient plasma and FVIII inhibitor plasma were done side by side. The results are shown in FIGS. **10**A and **10**B. As already shown in Example 6, there is a clear dose-dependent reduction of the clotting time in samples supplemented with antibody 193/AD3 in the presence of FVIII inhibitors.

Example 8

Anti-FIX/FIXa-antibodies are Procoagulant in the Presence of Defective FVIII and FIXa

To demonstrate that certain anti-FIXa-antibodies have FVIII-like activity in the presence of defective FVIII, the following experiment may be carried out. Increasing amounts of antibody 193/AD3 or, as a control, mouse IgG are used in a standard aPTT-based one stage clotting assay. In this clotting assay, a hemophilia A patient's plasma having very low clotting activity due to the presence of defective FVIII (DF8) is used. Briefly, 100 µl antibody samples are incubated with either 100 µl of DF8 plasma or FVIII deficient plasma as well as with 100 µl of DAPTTIN reagent, in a KC10A clotting analyzer. In addition, a total amount of 50 ng activated FIXa is included in the reaction mixture. After a short incubation, the reaction will be started by the addition of 100 µl $CaCl_2$ (25 mM). To ensure equal conditions, the experiment employing FVIII deficient plasma and DF8 plasma is done side by side.

Example 9

Anti-FIX/FIXa-antibodies with Procoagulant Activity in the Presence of FIXa Distinguish Between Human and Bovine FIXa

FIX/FIXa specific monoclonal antibodies selected from the 198th fusion experiment were purified from the respective hybridoma supernatant and quantified as described in Example 3. These antibodies were analyzed in a modified one-stage clotting assay (as described in Example 6) and some showed procoagulant activity.

The chromogenic activity of these antibody preparations was measured in the following FXa generation kinetic assay: 10 µg of monoclonal antibody (in 25 µl) were transferred to microtiter plate wells and warmed to 37° C. Chromogenic substrate (S-2222), synthetic thrombin inhibitor (I-2581), factor IXa and FX were reconstituted in sterile water and FIXa/FX (both bovine) were mixed with phospholipids according to the supplier's protocol. Per reaction, 50 µl of the phospholipid/FIXa/FX solution were combined with 25 µl $CaCl_2$ (25 mM) and 50 µl of the substrate/inhibitor cocktail. To start the reaction, 125 µl of the premix were added to the monoclonal antibody solution in the microtiter plates and incubated at 37° C. Absorbance at 405 nm and 490 nm of the samples was read at various times (5 min to 2 h) against a reagent blank (25 ml TBS instead of monoclonal antibodies) in a Labsystems iEMS Reader MF™ microtiter plate reader using GENESIS™ software. In parallel, the same reactions were performed except that 50 ng human FIXa were added per reaction. Those antibodies that showed procoagulant activity had no chromogenic activity in the case of bovine FIX, but displayed high activity when human FIXa was present.

FIG. **11** shows the time course of the FVIII-like activity exhibited by the monoclonal antibodies 198/A1, 198/B1 and 198/AP1 with (+) and without (−) addition of 50 ng human FIXaβ. Non-specific polyclonal mouse IgG was used as a control. 198/A1 and 198/B1 show procoagulant activity (similar as 193/AD3 in example 6) whereas 198/AP1 does not. Antibody 198/BB1 had the same activity pattern (data not shown).

Further monoclonal antibodies selected from the 198th fusion experiment include 198/Dl (ECACC NO. 99121616), 198/T2 (ECACC No. 99121617), 198/G2 (ECACC No.9912118), 198/U2 (ECACC No. 99121620).

Example 10

Structure and Procoagulant Activity of Antibody Derivatives Derived from Anti-FIX/FIXa-antibodies; Subcloning Antibody Variable Domains from Hybridoma Cell Lines 193/AD3, 193/K2, 198/A1 and 198/B1 (Clone AB2)

Cloning procedure: Messenger RNA was prepared from 1×10^{-6} hybridoma cells of the respective cell line (either 193/AD3, 193/K2, 198/A1 or 198/B1 (clone AB2)) employing the "QickPrep® Micro mRNA Purification Kit" (Pharmacia) according to the manufacturer's instructions. The corresponding cDNA was produced by retro transcription of mRNA using the "Ready-To-Go-You-Prime-First-Strand Beads kit" (Pharmacia) according to the manufacturer's instructions. Heavy and light chain encoding sequences were converted to the corresponding cDNA employing a set of primers. To reverse transcribe heavy chain-specific mRNA (VH), an equimolar mixture of the oligonucleotides MOCG1-2FOR (5' CTC AAT TTT CTT GTC CAC CTT GGT GC 3') (SEQ.ID.NO. 1), MOCG3FOR (5'CTC GAT TCT CTT GAT CAA CTC AGT CT 3') (SEQ.ID.NO. 2) and MOCMFOR (5' TGG AAT GGG CAC ATG CAG ATC TCT 3') (SEQ.ID.NO. 3) was used (RTmix1). In the same reaction tube, light chain-specific cDNA (VL) was synthesized using primer MOCKFOR-(5'CTC ATT CCT GTT GAA GCT CTT GAC 3') (SEQ.ID.NO. 4).

The coding sequences for VH were amplified by PCR using the primer-sets depicted in FIG. **12** and the specific cDNA, derived from the reverse transcription mixture (RTmix1) described above, as the template. VK-chain genes were amplified using the primer sets depicted in FIG. **13** and also employing Rtmix1 as a template. The VF-PCR product was cleaved SfiI-AscI and inserted into SfiI-AscI digested vector pDAP2 (GeneBank accession no.: U35316). The pDAP2-VH constructs obtained thereby were named pDAP2-193AD3/VH, pDAP2-198A1/VH, pDAP2-198AB2/VH (derived from antibody 198/B1) and pDAP2-193/K2/VH, respectively. The plasmids were subsequently cleaved with AscI-NotI and the corresponding AscI-NotI digested VK-gene PCR product was inserted. The resultant vectors were designated pDAP2-193/AD3scFv, pDAP2-198/A1scFv, pDAP2-198/AB2scFv (derived from antibody 198/B1) and pDAP2-193/K2scFv and code for the VH-gene and the VL-gene of the monoclonal antibodies 193/AD3, 198/A1, 198/AB2 (derived from antibody 198/B1) and 193/K2. Heavy and light chains are linked by the coding sequence for an artificial, flexible linker ($G_4SGGRASG_4S$ (SEQ ID NO:111); Engelhardt et al., 1994) and enables expression of the scFv variant of the respective antibody.

In FIG. **14**, the DNA and the deduced protein sequence of the scFv derived from the hybridoma cell line 193/AD3 are depicted. Nucleotides 1 to 357 code for the heavy chain variable domain, nucleotides 358 to 402 code for the artificial flexible linker and nucleotides 403 to 726 code for the light chain variable region. The protein sequence of the CDR3 region of the heavy chain has the sequence YGNSP-KGFAY (SEQ ID NO:5) and is given in bold letters. The artificial linker sequence ($G_4SGGRASG_4S$; SEQ ID NO:111) is shown.

In FIG. **15**, the DNA and the deduced protein sequence of the scFv derived from the hybridoma cell line 193/K2 is shown. Nucleotides 1 to 363 code for the heavy chain variable domain, nucleotides 364 to 408 code for the artificial flexible linker, and nucleotides 409 to 747 code for the light chain variable region. The protein sequence of the CDR3 of the heavy chain has the sequence DGGHGYGSS-FDY (SEQ ID NO:6), and is given in bold letters. The artificial linker sequence ($G_4SGGRASG_4S$; SEQ ID NO:111) is shown.

In FIG. **16**, the DNA and the deduced protein sequence of the scFv derived from the hybridoma cell line 198/AB2 (derived from antibody 198/B1) are depicted. Nucleotides 1 to 366 code for the heavy chain variable domain, nucleotides 367 to 411 code for the artificial flexible linker, and nucleotides 412–747 code for the light chain variable region. The protein sequence of the CDR3 region of the heavy chain has the sequence EGGGFTVNWYFDV (SEQ ID NO:7) and is given in bold letters. The artificial linker sequence ($G_4SGGRASG_4S$; SEQ ID NO:111) is also shown.

In FIG. **17**, the DNA and the deduced protein sequence of the scFv derived from the hybridoma cell line 198/A1 are depicted. Nucleotides 1 to 366 code for the heavy chain variable domain, nucleotides 367 to 411 code for an artificial flexible linker, and nucleotides 412–747 code for the light chain variable region. The protein sequence of the CDR3 region of the heavy chain has the sequence EGGGYYVN-WYFDV (SEQ ID NO:8) and is given in bold letters. The artificial linker sequence ($G_4SGGRASG_4S$; SEQ ID NO:111) is also shown.

Example 11

Procoagulant Activity of Peptides Derived from CDR3 Regions of Anti-FIX/FIXa-Antibodies

In principle, the antibody molecule can be envisioned as a biological device for the presentation of a combinatorial array of peptide elements in three dimensional space (see Gao et al., 1999, PNAS, 96:6025). Therefore, an antibody (or an antibody derivative, e.g. scFv, Fab, etc.) can be used either as a tool for the detection of functionally important domains of a specific target protein, or on the other hand, for the delineation of amino acid sequences specifically mediating certain interactions, i.e. activating or enhancing the activity of FIXa towards the physiological substrate FX. The latter process has led to the evaluation of a number of heavy chain CDR3 region (CDR3H) derived peptide sequences as FIXa enhancing agents.

Enhancing the procoagulant activity of peptides which exhibit such activity may be accomplished through sequence variation within the peptide regions critical for mediating the FIXa activity enhancement. As a possible step towards peptide sequences with enhanced procoagulant activity, the binding site of an antibody, i.e. 198/A1 or 198/B1, on the FIXa molecule is mapped by employing sequence comparison analyses, competitive binding assays, Western blot analyses and competitive ELISA analyses. Since the crystal structure of FIX is known, molecular modeling is subsequently used to improve the fitting of i.e. 198/B1 derived peptides in the 198/B1 binding site on human FIXa.

On the other hand, methodical mutational analysis of a given peptide sequence such as 198/A1 or 198/B1 CDR3H derived peptide sequences by, e.g., "alanine scanning mutational analysis" allows for the identification of peptide residues critical for procoagulant activity. Another way to improve the activity of a certain peptide sequence is the use of peptide libraries combined with high throughput screening.

The antigen binding site of an antibody is derived from the juxtaposition of the six "complement determining regions (CDR's)" at the N-terminal end of the VL-HL dimer

(or Fv region). The contribution of a single CDR to the antibody specificity for a given antigen may vary considerably, but in general it is thought that the CDR3 region of the heavy chain (CDR3H) is of special influence, i.e. the particular protein sequence of $CDR3_H$ region may be highly important for antigen recognition. The length of CDR3H regions has been reported to vary considerably and is in the range of 4–25 amino acids (Borrebaeck, p. 16).

An example of a methodical mutational analysis of peptide sequences is given below. To improve the solubility/procoagulant efficacy of peptides derived from the CD3-region of anti FIX/FIXa antibodies, the N-terminal as well as the C-terminal amino acid sequences were changed. In addition, a series of mutated peptides was constructed and analyzed.

The principle of such a study is exemplified by a series of peptides derived from CDR3H region of antibodies 198/A1 and 198/B1. The original peptide A1 (see table 2) is derived from the CDR3H region of antibody 198/A1 and peptide B1 is derived from the CDR3H region of antibody 198/B1, respectively (see example 10, FIGS. **16** and **17**). The term "scrambled version" means that a peptide has the same amino acids but in random order.

Peptide	Sequence	Amino-acids	MW (D)	pI	Remark
A1	EGGGYYVNWYFDV (SEQ ID NO:8)	(13aa)	1569	7.2	Decreased solubility
A1/1	VYGFGWGYEVNDY (SEQ ID NO:10)	(13aa)	1569	7.1	Scrambled version of A1,
A1/2	EEEEGGGYYVNWYFDEEE (SEQ ID NO:11)	(18aa)	2244	5.8	Acidic pI, soluble,
A1/3	RRREGGGYYVNWYFDRRR (SEQ ID NO:12)	(18aa)	2407	9.9	Basic pI, soluble,
A1/4	EYGEGYGEVNEYDEFEWE (SEQ ID NO:13)	(18aa)	2244	5.8	Scrambled version of A1/2
A1/5	VRYRNRYRWGYRGRFGDE (SEQ ID NO:14)	(18aa)	2407	9.9	Scrambled version of A1/3
A1/3-scr3	RRRGEYGVYWNGDFYRRR (SEQ ID NO:15)	(18aa)	2407	9.9	Scrambled version of A1/3
A1/3-Rd	RdRdRdEGGGYYVNWYFDRdRdRd	(18aa)	2407	9.9	Peptide A1/3 but substitute D-Arg for L-Arg
A1/3-Rd-srmb	RdRdRdGEYGVYWNGDFYRdRdRd	(18aa)	2407	9.9	Scrambled version of A1/3-Rd

Table 2

List of a series of antibody 198/A1 derived peptizes. Listed are the length of the peptide (aa, amino acids #), the calculated molecular weight (MW, in Dalton (D) and the statistical isoelectric point (pI).D-Arg is abbreviated as Rd.

In a first series of experiments we improved the solubility of the original CDR3H peptide sequence (A1; EGGGYYVNWYFDV; SEQ ID NO:8) by removing the C-terminal Val residue and adding several charged residues at the N— as well as the C-terminal end of the peptide. The resulting peptides, A1/2 (acidic pI), A1/3 (basic pI) and their respective scrambled versions A1/4, A1/5 and A1/3scr3 were readily soluble in a variety of buffer systems at physiological pH.

To analyze the FVIII-like (FIXa activating) activity of the peptides, an assay system based on a commercial available FVIII assay was developed (see examples 2 and 4). The basic principle is, that without a cofactor, FIXa will have very limited activity towards its natural substrate FX. Only in the presence of a substance having FIXa activation properties, i.e. FVIII or a substance exhibiting FVIII-like activity, a substantial amount of FXa is produced by cleavage of FX through the FIXa/activator complex. The amount of FXa generated is monitored by cleavage of a chromogenic substrate. The principle of the revised chromogenic assay is described for two representative peptides: A1/3 and A1/5 (Table 2). Briefly, 25 µl aliquots of peptide stock solution (in imidazole buffer (IZ) 50 mM imidazole, 100 mM NaCl, pH7.2) were transferred to microtiter plate wells and warmed to 37° C. Chromogenic FXa substrate (S-2222), synthetic thrombin inhibitor (I-2581), bovine FIXa and bovine FX were reconstituted in sterile water and FIXa/FX mixed with phospholipids according to the supplier's protocol. Since the peptides do not react with bovine FIXa, (which comes as a mixture with bovine FX in the Test Kit) 2,9 nM (in most cases 2.3 nM) human FIXa (ERL) were added (see Example 11, FIG. **19**). Per reaction, 50 µl of the phospholipid/FIXa/FX solution were combined with 251 $CaCl_2$ (25 mM) and 50 µl of the substrate/inhibitor cocktail. To start the reaction, 125 µl of the premix were added to the peptide solution in the microtiter plate and incubated at 37° C. Absorbance at 405 nm and 490 nm of the samples was read at various times (5 min to 2 h) against a reagent blank in a Labsystems iEMS Reader MF™ microtiter plate reader using GENESIS™ software.

The result of this experiment are shown in Example 11, FIG. **18**. Peptide A1/3 induced a readily measurable FXa generation in the presence of 2.9 nM human FIXa, whereas the scrambled version A1/5 was inactive. In addition, the acidic peptide A1/2 as well as the scrambled versions A1/4 and A1/3-scr3 did not give any significant chromogenic activity when tested under comparable conditions (data not shown). To prove that the peptide A1/3 like the parental antibody 198/A1 does not react with bovine FIXa and FX the experiment shown in FIG. **19** was done. The peptide

A1/3 was incubated as described above with (A1/3 (24 μM), +hFIXa) and without (A1/3 (24 μM), w/o hFIXa) 2.3 nM human FIXa (hFIXa). In a control experiment we added plain dilution buffer (IZ) supplemented with 2.3 nM hFIXa to the reaction mixture. As shown in FIG. **19**, the reaction takes place only in the presence of human FIXa.

FIG. **18** demonstrates the chromogenic FVIII-like activity of peptide A1/3 in the presence of 2.9 nM human FIXa (hFIXa). The scrambled version of peptide A1/3, peptide A1/5 does not give rise to any FXa generation. FIG. **19** demonstrates the dependence of the chromogenic FVIII-like activity of peptide A1/3 on the presence of human FIXa (hFIXa). In the absence of human FIXa, peptide A1/3 does not give rise to any FXa generation. The buffer control, plain imidazole buffer is designated IZ.

The peptides were also analyzed for their potential to reduce the clotting time in a FVIII deficient plasma. The aPTT based one stage clotting assay was essentially done as described (see example 6). Clotting times (time from starting the reaction to the "clot"-formation were compared either against FVIII, a buffer control (IZ) or a control peptide (scrambled version). The results of two typical clotting experiments done with two different aPTT reagents (DAPTTIN and Pathromtin SL) are shown in table 3A and table 3B.

	peptide conc.	w/o FIXa sec	w/o FIXa sec	average sec	2.2 nM FIXa sec	2.2 nM FIXa sec	average sec
Exp. 1							
IZ	0	107.7	106.8	107	93.1	94.5	94
A1/3	15 μM	78.2	77.1	78	59.3	59.9	60
	12.5 μM	80.2	80.6	80	60.2	58.9	60
	7.5 μM	97.8	97.9	98	73.1	72.7	73
	2.5 μM	105.2	104.8	105	91.1	91	91
A1/3-scr3	15 μM	122.5	122	122	106.1	105.5	106
	12.5 μM	116	117.6	117	103.1	104.5	104
	7.5 μM	114.2	113.9	114	100.8	100.6	101
	2.5 μM	107.8	107.4	108	96.3	95.2	96
Exp. 2							
IZ	0	111	109.7	110	94.7	95.5	95
A1/3	12.5 μM	83.6	85.5	85	56.7	56.7	57
	10 μM	79.1	78.5	79	63.1	62.5	63
	7.5 μM	100.1	100.5	100	71.6	73.9	73
	5 μM	103.4	104.8	104	77	76	77
	2.5 μM	110.1	108.9	110	88	88.8	88
	1.25 μM	108.7	109.3	109	90.7	90.8	91

Table 3A. Clotting activity of peptides A1/3 and A1/3-scr (scrambled version of A1/3) in FVIII deficient plasma either in the presence or in the absence (w/o) of 2.2 nM human FIXa. Shown are two independent representative experiments (Exp. 1 and Exp. 2). All clotting experiments have been done in duplicate. Given are the clotting times for the individual experiments and the average clotting time in seconds (sec). Experiments shown in table 3A have been done employing the aPTT reagent DAPTTIN (Baxter Hyland Immuno). Compared to the buffer control (IZ, imidazole buffer) the peptide A1/3 gave rise to a dose dependent reduction in the clotting time. The reduction in the clotting time became much more pronounced by the addition of 2.2 nM activated human FIX to the reaction mix. The scrambled version of peptide A1/3, A1/3-scr3 did not show any reduction of the clotting time. In fact, at concentrations above 2.5 μM, the scrambled peptide became inhibitory and therefore prolonged the clotting time. Peptides A1/1, A1/2, A1/4 and A1/5 did not give any reduction in the clotting time indicating that they lack procoagulant activity (data not shown).

	Final conc.	w/o FIXa sec	w/o FIXa sec	average sec	2.2 nM FIXa sec	2.2 nM FIXa sec	average sec
IZ	0	131.8	132.1	132	107.9	108.7	108
FVIII	12.5 mU/ml	68.9	69	69	52.9	53.6	53
	6.25 mU/ml	77.8	77.9	78	58.6	58.9	59
A1/3	15 μM	152.8	149.3	151	75.4	75.2	75
	10 μM	135.7	134.6	135	76.2	79.8	78
	5 μM	152.6	155.6	154	86.6	90.2	88
	1 μM	138.3	138.8	139	103.7	105.9	105

Table 3B. Clotting activity of peptide A1/3 in FVIII deficient plasma when Pathromtin SL (DADE Behring) is used as an aPTT reagent. The experiments were done in duplicate, either in the presence or in the absence (w/o) of 2.2 nM human FIXa. Given are the clotting times for the individual experiments and the average clotting time in seconds (sec). Factor VIII and imidazole buffer (IZ) were included as positive and negative control respectively.

In contrast to the experiments shown in table 3A the experiments shown in table 3B have been done employing the aPTT reagent Pathromtin SL. In the presence of FIXa, the peptide A1/3 gave rise to a dose dependent reduction in the clotting time whereas in the absence of FIXa no reduction of the clotting time was detectable.

In another series of experiments we set out to improve the plasma stability (protection from, e.g., proteolytic degradation) of peptide A1/3. One approach was to substitute the N- and C-terminal L-Arg residues with D-Arg residues (exemplified by peptides A1/3-rd and A1/3-Rd-srmb). Peptides A1/3-rd and A1/3-Rd-srmb (scrambled version of the peptide) were then analyzed in a chromogenic as well as in the aPTT based clotting assay. These experiments revealed that exchanging the terminal L-Arg residues for D-Arg residues did not change the FVIII-like activity as measured in the chromogenic assay, indicating that chirality of the Arg-residues does not play a major role in chromogenic activity (FIG. **20**). In addition, the aPTT based one-stage clotting activity, although somewhat reduced, was still easily detectable (Table 4).

	Peptide conc.	w/o FIXa sec	w/o FIXa, sec	average sec	2.2 nM FIXa sec	2.2 nM FIXa sec	average sec
IZ	0	110	109.1	110	96	96	96
A1/3	15 μM	77.8	78	78	56.1	55.5	56
	12.5 μM	99.4	100.5	100	65	68	67
	10 μM	104.4	104.5	104	72	73.2	73
	7.5 μM	105.2	105.2	105	80.7	80.5	81
	5 μM	108.4	107.7	108	89.7	88.3	89
	2.5 μM	107.9	107.6	108	93.6	93.3	93
	1.25 μM	106.7	107	107	94.4	95	95
A1/3-Rd	15 μM	96.4	95.4	96	76.1	74.4	75
	12.5 μM	98	98.6	98	72.3	73.7	73
	10 μM	93.5	95.8	95	74.2	77.2	76
	7.5 μM	97.6	98.1	98	80.9	82.2	82
	5 μM	99.2	99.1	99	86	85.1	86
	2.5 μM	102.7	103.4	103	94.4	94.7	95
	1.25 μM	107.5	107.7	108	96.6	96	96
A1/3-Rd srmb	15 μM	121.9	121.3	122	112.7	112.4	113
	12.5 μM	117.2	118	118	108.1	107.8	108
	10 μM	115.8	115.3	116	107.2	107.8	108
	7.5 μM	114.6	113.6	114	107.6	106.6	107
	5 μM	113.1	112.4	113	108.5	108.2	108

-continued

Peptide conc.	w/o FIXa sec	w/o FIXa, sec	average sec	2.2 nM FIXa sec	2.2 nM FIXa sec	average sec
2.5 μM	111.9	111.9	112	105	104.2	105
1.25 μM	107.2	107.1	107	101.1	105.3	103

Table 4. One stage clotting activity of peptides A1/3, A1/3-Rd and A1/3-Rd-srmb (sequences see table 2). IZ, buffer control.

FIG. **20** demonstrates the unchanged chromogenic activity of peptide A1/3-Rd. Peptides at a final concentration of 12 μM or the buffer control (IZ) were incubated in the presence of 2.3 nM human FIXa (+). The chromogenic activity of peptide A1/3 and A1/3-Rd was found to be virtually unchanged and gave almost identical results in the chromogenic assay. The scrambled version of peptide A1/3, A1/5 as well as the buffer gave no significant FXa generation.

In the next series of experiments we set out to determine the individual role of any amino acid of the peptide core sequence by substituting each residue for the amino acid Alanine (Table 5).

100 mM NaCl, 1% human albumin, pH7.4) to the desired final concentration. The peptides were analyzed for their chromogenic activity as well as for their potential to reduce the clotting time in a FVIII deficient plasma. The one-stage clotting assay was essentially done as described (see example 6). Clotting times (time from starting the reaction to the "clot"-formation were compared either against a buffer control or a control peptide (scrambled version).

Some of the results of the "Alanine scan" are given for the peptides A1/3-2 and A1/3-3. The change of G_3-A_3 as exemplified in the peptide A1/3-2 yields high chromogenic activity and a strong reduction of the one-stage clotting time (34 seconds at a concentration of 12.5 μM) in the presence of 2.2 nM human FIXa. Peptide A1/3-3 (G_4-A_4) exhibits an optimum of chromogenic activity around a final concentration of 12 μM with decreased activity at either higher or lower concentrations. The peptide is somewhat inhibitory in a one-stage clotting assay at higher concentrations (12.5 μM) in the absence of FIXa but becomes strongly active in the presence of 2.2 nM FIXa (31 seconds, 12.5 μM).

In the next series of experiments we set out to determine the individual role of any amino acid of the peptide core sequence by substituting each core residue for the amino acid glutamic acid (E) (see Table 6).

Peptide	Sequence	Amino acid #	MW (D)	pI	Remark
A1/3	RRREGGGYYVNWYFDRRR (SEQ ID NO:12)	(18aa)	2407	9.9	Basic pI, soluble.
A1/3-13	RRRAGGGYYVNWYFDRRR (SEQ ID NO:19)	(18aa)	2349	10.4	E_1–A_1
A1/3-1	RRREAGGYYVNWYFDRRR (SEQ ID NO:20)	(18aa)	2421	9.9	G_2–A_2
A1/3-2	RRREGAGYYVNWYFDRRR (SEQ ID NO:21)	(18aa)	2421	9.9	G_3–A_3
A1/3-3	RRREGGAYYVNWYFDRRR (SEQ ID NO:22)	(18aa)	2421	9.9	G_4–A_4
A1/3-4	RRREGGGAYVNWYFDRRR (SEQ ID NO:23)	(18aa)	2315	9.9	Y_5–A_5
A1/3-5	RRREGGGYAVNWYFDRRR (SEQ ID NO:24)	(18aa)	2315	9.9	Y_6–A_6
A1/3-6	RRREGGGYYANWYFDRRR (SEQ ID NO:25)	(18aa)	2379	9.9	V_7–A_7
A1/3-7	RRREGGGYYVAWYFDRRR (SEQ ID NO:26)	(18aa)	2364	9.9	N_8–A_8
A1/3-8	RRREGGGYYVNAYFDRRR (SEQ ID NO:27)	(18aa)	2292	9.9	W_8–A_9
A1/3-9	RRREGGGYYVNWAFDRRR (SEQ ID ND:28)	(18aa)	2315	9.9	Y_{10}–A_{10}
A1/3-10	RRREGGGYYVNWYADRRR (SEQ ID NO:29)	(18aa)	2331	9.9	F_{11}–A_{11}
A1/3-11	RRREGGGYYVNWYFARRR (SEQ ID NO:30)	(18aa)	2363	10.5	D_{12}–A_{12}
A1/3-12srmb	RRRYVYNGWGYFEGARRR (SEQ ID NO:31)	(18aa)	2363	10.4	Scrambled version

Table 5. Listed are the peptides designed to elucidate the role of any single amino acid within the peptide core sequence ($E_1G_2G_3G_4Y_5Y_6V_7N_8W_9Y_{10}F_{11}D_{12}$; SEQ ID NO:112). The subscripted numbers describe the position of the amino acid within the peptide. Alanine, an uncharged small amino acid, was substituted for each amino acid ("Alanine scan"). Also listed are the lengths of the peptides (amino acids #), the calculated molecular weights (MW, in Dalton (D) and the statistical isoelectric points (pI).

Each of the peptides was dissolved individually in imidazole buffer (50 mM imidazole, 100 mM NaCl, pH7.2) and subsequently diluted in clotting buffer (50 mM imidazole,

Peptide	Sequence	Amino-Acids	MW (D)	pI	Remark
A1/3	RRREGGGYYVNWYFDRRR (SEQ ID NO:12)	(18aa)	2407	9.9	Basic pI, soluble,
A1/3-22	RRREEGGYYVNWYFDRRR (SEQ ID NO:32)	(18aa)	2479	9.5	G_2–E_2
A1/3-23	RRREGEGYYVNWYFDRRR	(18aa)	2479	9.5	G_3–E_3

-continued

Peptide	Sequence	Amino-Acids	MW (D)	pI	Remark
	(SEQ ID NO:33)				
A1/3-24	RRREGGEYYVNWYFDRRR (SEQ ID NO:34)	(18aa)	2479	9.5	G_4–E_4
A1/3-26	RRREGGGEYVNWYFDRRR (SEQ ID NO:35)	(18aa)	2373	9.4	Y_5–E_5
A1/3-27	RRREGGGYEVNWYFDRRR (SEQ ID NO:36)	(18aa)	2373	9.4	Y_6–E_6
A1/3-28	RRREGGGYYENWYFDRRR (SEQ ID NO:37)	(18aa)	2437	9.5	V_7–E_7
A1/3-29	RRREGGGYYVEWYFDRRR (SEQ ID NO:38)	(18aa)	2422	9.5	N_8–E_8
A1/3-30	RRREGGGYYVNEYFDRRR (SEQ ID NO:39)	(18aa)	2350	9.5	W_9–E_9
A1/3-31	RRREGGGYYVNWEFDRRR (SEQ ID NO:40)	(18aa)	2373	9.4	Y_{10}–E_{10}
A1/3-32	RRREGGGYYVNWYEDRRR (SEQ ID NO:41)	(18aa)	2389	9.5	F_{11}–E_{11}
A1/3-33	RRREGGGYYVNWYFERRR (SEQ ID NO:42)	(18aa)	2421	9.9	D_{12}–E_{12}
A1/3-34srmb	RRRGEYGEYWNGDFYRRR (SEQ ID NO:43)	(18aa)	2437	9.5	Scrambled version

Table 6. Listed are the peptides designed to elucidate the role of any single amino acid within the peptide core sequence ($E_1G_2G_3G_4Y_5Y_6V_7N_8W_9Y_{10}F_{11}D_{12}$; SEQ ID NO:112). The subscripted numbers describe the position of the amino acid within the peptide. Glutamic acid, a negatively charged large amino acid, was substituted for each amino acid of the core sequence ("Glutamic acid scan"). Also listed are the lengths of the peptide (amino acids #), the calculated molecular weights (MW, in Dalton (D) and the statistical isoelectric points (pI).

Each of the peptides was solved individually in imodazole buffer (50 mM imidazole, 100 mM NaCl, pH7.2) and subsequently diluted in clotting buffer (50 mM imidazole, 100 mM NaCl, 1% human albumin, pH7.4) to the desired final concentration. The peptides derived from the "Glutamic acid scan" series were analyzed for their chromogenic FVIII-like activity as well as for their potential to reduce the clotting time in a FVIII deficient plasma. The one-stage clotting assay was essentially done as described (see example 6).

The peptide A1/3-24 showed some interesting properties. The molecule exhibited high chromogenic FVIII-like activity at concentrations between 6.5 μM-12 μM but lost activity at higher concentrations (up to 24 μM). The peptide had no procoagulant activity in the absence of human FIXa but was strongly active in the presence of 2.2 nM hFIXa.

In a second series of experiments we set out to improve the procoagulant activity of the antibody-198/B1 CDR3H derived peptide sequence B1. In a first step we improved the solubility of the original peptide sequence (B1; EGGGFTVNWYFDV; SEQ ID NO:7) by removing the C-terminal Val residue and adding several charged residues at the N- as well as the —C-terminal end of the peptide. The resulting peptides B1/4, B1/6 (acidic pI), B1/7 (basic pI) and their scrambled versions B1/5, B1/7scr3 are readily soluble in a variety of buffer systems at physiological pH.

Peptide	Sequence	Amino-acids	MW (D)	pI	Remark
B1	EGGGFTVNWYFDV (SEQ ID NO:7)	(13aa)	1491	6.0	Decreased solubility
B1/4	REGGGFTVNWYFDR (SEQ ID NO:45)	(14aa)	1704	7.9	Soluble,
B1/5	FGVGYRGETRNFDW (SEQ ID NO:46)	(14aa)	1704	8.0	Scrambled version, soluble
B1/6	EEEEGGGFTVNWYFDEEE (SEQ ID NO:47)	(18aa)	2166	5.0	Acidic pI soluble
B1/7	RRREGGGFTVNWYFDRRR (SEQ ID NO:48)	(18aa)	2329	9.9	Basic pI soluble
B1/7 scr3	RRRFGVGYGETNFDWRRR (SEQ ID NO:49)	(18aa)	2329	9.9	Basic pI, soluble, scrambled version

Table 7 is a list of a series of antibody 198/B1 derived peptides. Listed are the length of the peptide (aa, amino acids #), the calculated molecular weight (MW, in Dalton (D) and the statistical isoelectric point (pI).

Peptides B1/4 and B1/5 were soluble in 50 mM Tris, 100 mM NaCl, pH=6.5. Both peptides were analyzed in a chromogenic FVIII assay. Peptide B1/4 but not the scrambled version B1/5 was found to have some chromogenic activity (data not shown).

Subsequently peptides B1/6, B1/7 and B1/7scr3 were analyzed. Each of the peptides was solved individually in 50 mM imidazole, 100 mM NaCl, pH7.2 and subsequently diluted either in clotting buffer (50 mM imidazole, 100 mM NaCl, 1% human albumin, pH7.4) or in imidazole buffer to the desired final concentration. The peptides were analyzed for their chromogenic activity as well as for their potential to reduce the clotting time in a FVIII deficient plasma (table 8 & 9). The one stage clotting assay was essentially done as described (see example 6). Clotting times (time from starting the reaction to the "clot"-formation were compared either against a buffer control or a control peptide (scrambled version).

The FIXa activating activity (FVIII cofactor-like activity) from peptide B1/7 was first measured in the chromogenic assay described above.

As shown in FIG. **21**, the addition of 2.4 μM peptide B1/7 to the reaction mixture led to a well measurable generation of FXa. In contrast, the addition of 35 μM Pefabloc Xa, a specific inhibitor of FXa protease activity, resulted in a significant reduction of the chromogenic substrate cleavage reaction (FIG. **22**) thereby proving that there was indeed a peptide-FIXa mediated FXa generation. If there was no addition of FIXa and FX to the reaction mixture, no FXa was synthesized (FIG. **22**). Peptide B1/6 and the control peptides B1/5 and B1/7scr3 exhibited no activity (data not shown).

FIG. **21** demonstrates the chromogenic activity of peptide B1/7. The peptide at a final concentration of 2.4 μM or the buffer control (IZ) were incubated in the presence of 2.3 nM human FIXa.

In FIG. **22** peptide B1/7 at a final concentration of 2.4 μM or the buffer control (IZ) were incubated in the presence of 2.3 nM human FIXa (as indicated either as "+2.3 nM hFIXa" or "+") The chromogenic activity of peptide B1/7 was found to be dependent on the presence of FIXa and FX since no reaction is detectable when FIXa and FX are left out of the reaction (w/o FIXa/FX). To prove that the peptide B1/7 mediates indeed FXa generation, the FXa specific protease inhibitor Pefabloc Xa was added to the reaction mix (35 μM Pefabloc Xa). In a second set of experiments, the procoagu-

lant effect of peptides B1/6, B1/7 and B1/7scr3 were tested in a aPTT based one-step coagulation assay. The experiments were done essentially as described in Example 6. The results are shown in tables 8 and 9.

Peptide	12.5 µM (−)	1.25 µM (−)	0.125 µM (−)	12.5 nM (−)	Buffer (−)	remarks
B1/6	115	110	111	111	110	
B1/7	157	112	109	110	110	
B1/7 scr3	115	105	106	105	107	

Table 8: FVIII deficient plasma was incubated either with peptides B1/6, B1/7scr3 or B1/7 in the absence of activated human FIX. As a negative control, plain buffer was added to the deficient plasma. The clotting times for the various combinations are given. Under these conditions, peptide B1/7 at its highest concentration (12.5 µM) becomes inhibitory to the coagulation process as indicated by the extended clotting time of 157 seconds.

Peptide	12.5 µM (+)	1.25 µM (+)	0.125 µM (+)	12.5 nM (+)	Buffer (+)	remarks
B1/6	103	100	101	100	100	
B1/7	83	92	99	99	100	
B1/7 scr3	102	94	94	94	94	

Table 9: FVIII deficient plasma was incubated either with peptides B1/6, B1/7scr3 or B1/7 in the presence of activated human FIX. As a negative control, plain buffer was added to the deficient plasma. The clotting times for the various combinations are given. In the presence of FIXa, peptide B1/7 becomes procoagulant as indicated by the reduced clotting time (83 seconds compared to 102 seconds for the scrambled peptide and 100 seconds for the buffer control).

Example 12

Procoagulant Activity of Peptide Derivatives Obtained from CDR3 Regions of Anti-FIX/FIXa-Antibodies in FVIII Inhibitor Plasma

To assay for the procoagulant activity of peptide A1/3 in FVIII inhibitor plasma the following experiment was carried out. We performed a standard aPTT based one stage clotting assay, but instead of FVIII deficient plasma we employed FVIII inhibitor plasma. The inhibitory potency of the plasma was 8.1 Bethesda Units per ml.

TABLE 10

	Peptide conc.	w/o FIXa sec	w/o FIXa sec	Average sec	FIXa sec	FIXa sec	average sec
IZ	0	104.9	103.6	104	94.2	94.1	94
A1/3	12.5 µM	85.8	85.3	86	61	60.2	61
	10 µM	88.4	87.9	88	61.3	61.8	62
	7.5 µM	93.7	92.7	93	68.8	70.9	70
	5 µM	101.5	101.1	101	81	82	82
	2.5 µM	106.1	105.3	106	90.2	90.5	90
	1.25 µM	104.5	104.3	104	91.3	91.4	91

Table 10: Various amounts of peptide A1/3 (12.5 µM-1.25 µM) were added to FVIII inhibitor plasma (either in the presence (FIXa) of 2.2 nM FIXa or in the absence (w/o FIXa). As a negative control, plain buffer was added to the plasma (IZ). Experiments were done in duplicate and the average (aver.) was calculated. The clotting times (in seconds) for the various combinations are given. It is easily appreciable that the peptide A1/3 reduces (in a dose dependent manner) the clotting time of FVIII inhibitor plasma in the presence of FIXa but, although albeit to a much lesser extent, also in the absence of FIXa.

Example 13

Conversion of the 196/C4 IgM into IgG1

Since some IgM antibodies demonstrate high FVIII-like activity in chromogenic assays, attempts were made to convert such IgM antibodies into IgG antibodies (though antibody derivatives such as Fab, $F(ab)_2$, scFv, etc. could also be produced). Described in detail below is the rescue of the IgM variable region genes. Expression vector pBax-IgG1 (FIG. **23**) was first constructed from vectors pSI (Promega) and pEF/Bsd (Invitrogen) through multiple cloning steps. B-lymphocytes of a donor are purified from blood and mature mRNA purified from these cells using the "micro-mRNA purification-kit" (Pharmacia). The cDNA of a human kappa chain and a human gamma 1 chain are prepared employing the "you-primefirst-strand-cDNA-"kit" (Pharmacia) using specific primers.

The coding sequence of a human kappa light chain constant domain is amplified from the cDNA by PCR using specific primers.

The gene of a human gamma 1 chain constant region (CH1-hinge-CH2—CH3) is amplified from the cDNA by PCR using specific primers.

The PCR product of the light chain constant domain is digested with XbaI and NheI and inserted into digested pSI. The resultant vector is cleaved with EcoRI and XbaI and annealed oligonucleotides are inserted, resulting in vector pSI-Ckappa. The annealed oligonucleotides provide for the leader and the SacI-XbaI sites for insertion of the kappa chain variable region. The PCR product of the human gamma 1 chain constant region is digested with SpeI and BamHI and inserted into digested pSI. The resultant vector is cleaved with SpeI and NotI and annealed oligonucleotides are inserted resulting in vector pSI-Cgamma. The annealed oligonucleotides provide for the leader and the XhoI-BstEI sites for insertion of the heavy chain variable region. Vector pEF/Bsd is digested NheI and SfiI, blunt ended by Klenow treatment and the whole expression cassette of pSI-Ckappa, excised with BglII and BamHI, is inserted (after Klenow treatment). The resultant vector is digested with EcoRI and HindIII and treated with Klenow. The whole expression cassette of pSI-Cgamma is excised with BglII and BamHI and is inserted (after Klenow treatment). The resultant vector is named pBax-IgG1.

The light chain variable region can be inserted in between the SacI-XbaI sites, yielding the complete coding-sequence of a kappa light chain. The heavy chain variable region can be cloned in between the XhoI-BstEI sites, resulting in a complete IgG1 heavy chain gene. Both open reading frames are expressed under the control of the SV40-promoter and harbour the coding sequence of a signal peptide at the 5' end of the genes for secretion of the heavy and light chains into the endoplasmatic reticulum. Transfection into COS cells allows the expression of an IgG1 with the same binding properties as the parental IgM.

Construction of the plasmid pBax-196/C4 is further accomplished by amplifying the VH of the 196/C4 scFv

(subcloned as described in Experiment 10) by PCR using specific primers. The PCR product is digested with XhoI and BstEII and inserted into XhoI and BstEII digested pBax IgG1. The VL of the 196/C4 scFv is amplified by PCR using specific primers. The PCR product is digested with SacI and XbaI and inserted into SacI and XbaI-digested pBax IgG1-VH. The resultant vector (pBax-196/C4) is transfected into COS cells by electroporation, and hybrid IgG1 molecules (murine variable region and human constant region) with the same specificity as the parental IgM is expressed.

Example 14

Activation of FIXa Amydolytic Activity by Anti-FIXa Antibodies

Briefly, 20±1 factor IXa (containing 20 mU FIXa (Stago)) were incubated at 37° C., with 200 µl of reaction buffer (50 mM Tris HCl pH7.4, 100 mM NaCl, 5 mM $CaCl_2$ and 40% Ethyleneglycol), 25 µl of FIXa substrate (CH_3SO_2-D-CHG-Gly-Arg-pNA, AcOH, 10M/ml, Pentapharm LTD) in the absence or presence of various amounts of anti-FIX antibodies 198/B1 (IgG isotype) or 196/AF1 (IgM isotype). Specific cleavage of FIXa substrate was monitored at 405 nm in an ELISA reader.

The presence of the anti-FIX antibodies enhanced the amydolytic activity of FIXa at least 2 fold. FIG. **24** shows the increase of the amidolytic activity of FIXa in the presence of antibody 198/B1 (FIG. **24**A) and antibody 198/AF1 (FIG. **24**B).

Example 15

FVIII-like Activity Exhibited by Fab Fragments Derived from Anti FIX/FIXa-antibodies

Fab fragments of anti-FIX/FIXa antibodies were prepared and purified according to standard protocols. Briefly, 1 ml antibody 198/A1(4 mg/ml in 50 mM imidazole, 100 mM NaCl, pH7.4) was incubated overnight with 87 µl fragmentation buffer (1M Na Acetate, 10 mM EDTA 67.5 mg/ml L-cysteine) and 0.25 mg papain (immobilized on agarose beads), at 37° C. The preparation was filtered to remove the papain. L-histidine was added (final concentration 50 mM) and afterwards the pH was adjusted to 7.0. Finally, solid NaCl is added to give a final concentration of 1M.

Subsequently, the 198/A1 Fab fragment was purified by binding to protein L: we used ImmunoPure Immobilized PROTEIN L Plus (Pierce) in a PHARMACIA XK 16/20 Column (gel-volume: 2 ml) Buffers for chromatography were: 1) equilibration-buffer: 50 mM L-histidine pH 7.0; 1M NaCl; 0,1% (w/v) NaN_3; 2) wash-buffer: 50 mM L-Histidine pH 7.0; 0.1 (w/v) NaN_3; 3) elution-buffer: 100 mM glycine pH 2.5; 0.1% (w/v) NaN_3; and 4) neutralization buffer: 2M Tris/Cl pH 8,0;

Chromatography was essentially done by following steps 1 to 7 described in table 11. In order to neutralize the low pH of the elution buffer "Fraction-tubes" were pre-loaded with 0.2 ml 2M Tris pH 8.0.

TABLE 11

	STEP	BUFFER	Flow rate	Vol.	CV	Fractions
1.	column-wash	elution-buffer	2.0 ml/min	10 ml	5	waste
2.	equil-ibration	equi-buffer	2.0 ml/min	10 ml	5	waste
3.	sample-load	sample	1.0 ml/min	x ml	x	flow-through
4.	wash 1	equi-buffer	1.0 ml/min	20 ml	10	flow-through
5.	wash 2	wash-buffer	1.0 ml/min	10 ml	5	flow-through
6.	elution	elution-buffer	1.0 ml/min	15 ml	7.5	1,0 ml fractions-
7.	neutral-ization	wash-buffer	2.0 ml/min	10 ml	5	waste

Table 11

The final 198/A1 Fab preparation was dialyzed against 50 mM imidazole, 100 mM NaCl, pH7.4 and analyzed in a chromogenic FVIII assay as described above (FIG. **25**). Compared to an intact antibody, the 198/A1 Fab fragment has somewhat less activity; however, the Fab fragment still gives rise to FIX dependent FXa generation.

FIG. **25** demonstrates the chromogenic FVIII-like activity of the antibody 198/A1 Fab fragment in the presence of 2.3 nM human FIXa. As a positive control we used the intact antibody 198/A1 as well as 7.5 pM FVIII. Buffer control (IZ) instead of 198/A1 Fab fragment or FVIII was used as a negative control.

Example 16

FVIII-like Activity Exhibited by Fusion Proteins Between scFv Fragments of Anti-FIX/FIXa Antibodies and *E. coli* Alkaline Phosphatase

The single chain Fv fragment (see example 10) of antibody 198/B1 (subclone AB2) was fused to the N-terminus of *E. coli* alkaline phosphatase employing the pDAP2 vector system (Kerschbaumer et al., 1996). Two identical clones were isolated and designated pDAP2-198AB2#1 and pDAP2-198AB2#100 (FIG. **26**). The resulting fusion proteins were expressed in *E. coli*, purified by metal affinity chromatography (Kerschbaumer et al., 1997) and analysed in a standard chromogenic assay (FIG. **27**).

FIG. **27** demonstrates the chromogenic FVIII-like activity of two antibody 198/B1 (subclone AB2) scFv fragment-alkaline phosphatase fusion proteins (198AB2#1 and 198AB2#100) in the presence of 2.3 nM human FIXa. As a positive control we used 7.5 pM FVIII.

Example 17

FVIII-like Activity Exhibited by a Bivalent Miniantibody

In order to obtain a bivalent miniantibody, the scFv fragment of antibody 198/B1 (subclone AB2) was fused to a amphipatic helical structure employing the pZip1 vector system (Kerschbaumer et al. (Analytical Biochemistry 249, 219–227, 1997). Briefly, the gene of the 198/B1 scFv fragment was isolated from the plasmid pDAP-198AB2#100 (example 16) by digestion with SfiI and NotI. The DNA fragment was gel purified and inserted in the SfiI/NotI digested vector pZip1. The resulting plasmid was sequenced and designated pZip-198AB2#102 (FIG. **28**). In parallel, we constructed a miniantibody version from an irrelevant monoclonal antibody termed #8860. In a first step, the single chain Fv fragment of antibody #8860 was assembled in the vector pDAP2. The cloning was done essentially as described in example 10. The construct was

named pDAP2-8860scFv#11 (FIG. **29**). Subcloning of the scFv fragment contained within pDAP2-8860scFv#11 into plasmid pZip1 (see above) yielded the miniantibody construct p8860-Zip#1.2 (FIG. **30**). Since antibody #8860 does not react with FIX/FIXa (as judged by Western Blot and ELISA analysis) it represents an appropriate negative control. Subsequently, the miniantibody proteins were expressed in *E. coli* and purified from bacterial supernatants by binding to Protein L according to the following protocol: For affinity chromatography we used ImmunoPure Immobilized PROTEIN L Plus (Pierce) in a PHARMACIA XK 16/20 Columns having a gel-volume of 4 ml Buffers employed were: 1) equilibration-buffer: 5 mM L-Histidine pH 7.0, 1M NaCl, 0.1% (w/v) NaN_3, wash-buffer: 50 mM L-histidine pH 7.0, 0.1% (w/v) NaN_3; elution-buffer: 100 mM glycine pH 2.5, 0.1% (w/v) NaN_3; and neutralization buffer: 2M Tris/Cl pH 8.0.

Samples were prepared as follows: The bacterial culture supernatant was obtained by centrifugation of the bacterial expression culture (11,000×g, 4° C., 10 minutes). 470 g of ammonium-sulphate was added to 1 liter of supernatant and the solution stirred on ice for 1 hour to precipitate the protein. The precipitate was pelleted at 14,000×g for 35 minutes at 2° C. and re-dissolved in 100 ml 20 mM Tris pH 7.0. Subsequently the concentrate was dialyzed against 20 mM Tris pH 7.0, L-histidine was added to a final concentration of 50 mM and the pH was adjusted to 7.0. Finally, solid NaCl was added to give a final concentrations of 1M. Before loading on the column, a sample was first centrifuged at 16,000×g for 15 min at room temperature and then filtered through a 0.45 μm sterile filter.

Chromatography was essentially done by following steps 1 to 7 described in table 12. In order to neutralize the low pH of the elution buffer "Fraction-tubes" were pre-loaded with 0.2 ml 2M Tris pH 8.0.

	STEP	BUFFER	Flow rate	Vol.	CV	Fractions
1.	column-wash	elution-buffer	2.0 ml/min	20 ml	5	waste
2.	equil-ibration	equi-buffer	2.0 ml/min	20 ml	5	waste
3.	sample-load	sample	1.0 ml/min	x ml	x	flow-through
4.	wash 1	equi-buffer	1.0 ml/min	40 ml	10	flow-through
5.	wash 2	wash-buffer	1.0 ml/min	20 ml	5	flow-through
6.	elution	elution-buffer	1.0 ml/min	30 ml	7.5	1.0 ml fractions-
7.	neutral-ization	wash-buffer	2.0 ml/min	20 ml	5	waste

Table 12. The final 198/B1 (subclone AB2) miniantibody preparation (designated 198AB-Zip#102) and the negative control 8860-Zip#1.2 were dialyzed against 50 mM imidazole, 100 mM NaCl, pH7.4 and analyzed in a chromogenic FVIII assay as described above (FIG. **31**).

As can be seen in FIG. **31**, the miniantibody construct 198AB-Zip#102 gives rise to substantial FXa generation (compare to FVIII) whereas the negative control miniantibody 8860-Zip#1.2 does not.

FIG. **31** demonstrates the chromogenic FVIII-like activity of the 198/B1 (subclone AB2) miniantibody 198AB-Zip#102 in the presence of 2.3 nM human FIXa. As a positive control we used 4.8 pM FVIII whereas an unrelated miniantibody (8860-Zip#1.2) and plain reaction buffer (IZ) served as negative controls.

Example 18

FVIII-like Activity Exhibited by Anti-FIXa/FIX Antibody scFv Fragments

The single chain Fv fragment of antibody 198/B1 (subclone AB2) as well as the scfv fragment of antibody #8860 were expressed employing the pMycHis6 vector system. Vector pMycHis6 (FIGS. **32** & **33**) was constructed by cleaving vector pCOCK (Engelhardt et al., 1994, Biotechniques, 17: 44–46) with NotI and EcoRI and insertion of the following oligonucleotides: mychis6-co: 5' ggccgcagaacaaaaactcatctcagaagaggatct gaatggggcggcacatcaccatcaccatcactaataag 3' (SEQ. ID.NO.

79) and mycchis-ic: 5'aattcttattagtgatggtgatggtgatgtgccgccccattcagatcctct tctgagatgagtttttgttctgc 3' (SEQ.ID.NO. 80) FIG. **32** shows a schematic representation of the plasmid pMycHis6. The c-myc-tag sequence is used to detect the scFv fragment in an ELISA or a Western Blot analysis (Evan et al., Mol.Cell.Biol., 1985, 5(12), pp. 3610–6). The His6-tag sequence was included to facilitate the purification of scFv fragments by metal ion chromatography (Hochuli et al., 1988. Biotechnology, 6: 1321–1325). The plasmid contains the lacZ gene promoter (PlacZ) the PelB-leader sequence (see legend FIG. **26**) an *E. coli* origin of replication (colElori) and a M13 phage origin of replication (M13ori). To allow for specific selection, the plasmid also carries the gene for the enzyme β-lactamase (AmpR) mediating resistance against the antibiotic ampicillin.

The gene of the 198/B1 (clone AB2)-scFv was rescued from plasmid pDAP2-198AB2#100 (example 16) by digestion with SfiI and NotI and inserted into SfiI/NotI cleaved pMycHis6. The resultant plasmid was designated pMycHis-198AB2#102. FIG. **34** shows the nucleotide and amino acid sequence of 198AB2 scFv (linked to the c-myc-tag and the His6tag):the resulting ORF of the expression vector is named pMycHisG-198AB2#102. Vector pMycHis6 was constructed by cleaving vector pCOCK (Engelhardt O. et al, BioTechniques 17, 44–46, 1994) NotI-EcoRI and inserting the following annealed oligonucleotides: (5'-GGCCGCAGAACAAAAACTCATCTCAGAAGAGGATCTGAATGGG GCGGCACATCACCATCACCATCACTAATAAG-3' (SEQ.ID.No. 103) and 5'-TTATTAGTGATGGTGATGGTGATGTGCCGCCCCATTCAGATCCTCTTCTGAGATGAGTTTTTGTTCTGC-3'(SEQ.ID.NO. 104)). The resultant vector, named pMycHis6, was cleaved SfiI-NotI and the gene of scFv 198AB2 was swapped into this vector from vector pDAP2-198AB2#100.

In analogy to the 198AB2 construct, the #8860 scFv fragment was cloned from a plasmid designated pDAP2-8860scFv clone 11. The pure scFv protein of #8860 was designated 8860-M/H#4c (plasmid p8860-M/H#4c, FIG. **35**). The scFv proteins were expressed in *E. coli* and affinity purified from bacterial supernatants on Protein L columns (see example 17). The final MycHis-198AB2#102 and 8860-M/H#4c preparations were dialyzed against 50 mM imidazole, 100 mM NaCl, pH7.4 and analyzed in a chromogenic FVIII assay as described above (FIG. **36**).

As can be seen in FIG. **36**, the scfv construct MycHis-198AB2#102 gave rise to a substantial FXa generation whereas the negative controls 8860-M/H#4c and plain reaction buffer (IZ) did not.

FIG. **36** demonstrates the chromogenic FVIII-like activity of the 198/B1 (subclone AB2) scFv fragment (MycHis-198AB2#102) in the presence of 2.3 nM human FIXa. As a positive control we used 4.8 pM FVIII whereas a unrelated scfv (8860-M/H#4c) and plain reaction buffer (IZ) served as negative controls.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 112

<210> SEQ ID NO 1
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:primer oligonucleotide MOCG1-2FOR

<400> SEQUENCE: 1

ctcaattttc ttgtccacct tggtgc 26

<210> SEQ ID NO 2
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:primer oligonucleotide MOCG3FOR

<400> SEQUENCE: 2

ctcgattctc ttgatcaact cagtct 26

<210> SEQ ID NO 3
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:primer oligonucleotide MOCMFOR

<400> SEQUENCE: 3

tggaatgggc acatgcagat ctct 24

<210> SEQ ID NO 4
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:primer MOCKFOR

<400> SEQUENCE: 4

ctcattcctg ttgaagctct tgac 24

<210> SEQ ID NO 5
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:hybridoma cell line 193/AD3 heavy chain CDR3 region

<400> SEQUENCE: 5

Tyr Gly Asn Ser Pro Lys Gly Phe Ala Tyr
1 5 10

<210> SEQ ID NO 6
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:hybridoma cell line 193/K2 heavy chain CDR3 region

-continued

<400> SEQUENCE: 6

Asp Gly Gly His Gly Tyr Gly Ser Ser Phe Asp Tyr
1 5 10

<210> SEQ ID NO 7
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:hybridoma cell line 193/AB2 (derived from antibody 198/B1) heavy chain CDR3 region, peptide B1

<400> SEQUENCE: 7

Glu Gly Gly Gly Phe Thr Val Asn Trp Tyr Phe Asp Val
1 5 10

<210> SEQ ID NO 8
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:hybridoma cell line 198/A1 heavy chain CDR3 region, peptide A1

<400> SEQUENCE: 8

Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Val
1 5 10

<210> SEQ ID NO 9

<400> SEQUENCE: 9

000

<210> SEQ ID NO 10
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutated peptide A1/1 scrambled versioon of A1

<400> SEQUENCE: 10

Val Tyr Gly Phe Gly Trp Gly Tyr Glu Val Asn Asp Tyr
1 5 10

<210> SEQ ID NO 11
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutated peptide A1/2

<400> SEQUENCE: 11

Glu Glu Glu Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Glu
1 5 10 15

Glu Glu

<210> SEQ ID NO 12
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutated peptide A1/3

-continued

<400> SEQUENCE: 12

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 13
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutated peptide A1/4 scrambled version of A1/2

<400> SEQUENCE: 13

Glu Tyr Gly Glu Gly Tyr Gly Glu Val Asn Glu Tyr Asp Glu Phe Glu
1 5 10 15

Trp Glu

<210> SEQ ID NO 14
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutated peptide A1/5 scrambled version of A1/3

<400> SEQUENCE: 14

Val Arg Tyr Arg Asn Arg Tyr Arg Trp Gly Tyr Arg Gly Arg Phe Gly
1 5 10 15

Asp Glu

<210> SEQ ID NO 15
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutated peptide A1/3-scr3 scrambled version of A1/3

<400> SEQUENCE: 15

Arg Arg Arg Gly Glu Tyr Gly Val Tyr Trp Asn Gly Asp Phe Tyr Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 16

<400> SEQUENCE: 16

000

<210> SEQ ID NO 17

<400> SEQUENCE: 17

000

<210> SEQ ID NO 18

<400> SEQUENCE: 18

000

<210> SEQ ID NO 19

-continued

<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-13 Alanine scan E-1-A-1

<400> SEQUENCE: 19

Arg Arg Arg Ala Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 20
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-1 Alanine scan G-2-A-2

<400> SEQUENCE: 20

Arg Arg Arg Glu Ala Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 21
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-2 Alanine scan G-3-A-3

<400> SEQUENCE: 21

Arg Arg Arg Glu Gly Ala Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 22
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-3 Alanine scan G-4-A-4

<400> SEQUENCE: 22

Arg Arg Arg Glu Gly Gly Ala Tyr Tyr Val Asn Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 23
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-4 Alanine scan Y-5-A-5

<400> SEQUENCE: 23

Arg Arg Arg Glu Gly Gly Gly Ala Tyr Val Asn Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

-continued

<210> SEQ ID NO 24
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-5 Alanine scan Y-6-A-6

<400> SEQUENCE: 24

Arg Arg Arg Glu Gly Gly Gly Tyr Ala Val Asn Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 25
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-6 Alanine scan V-7-A-7

<400> SEQUENCE: 25

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Ala Asn Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 26
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-7 Alanine scan N-8-A-8

<400> SEQUENCE: 26

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Ala Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 27
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-8 Alanine scan W-9-A-9

<400> SEQUENCE: 27

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Asn Ala Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 28
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-9 Alanine scan Y-10-A-10

<400> SEQUENCE: 28

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Asn Trp Ala Phe Asp Arg
1 5 10 15

Arg Arg

-continued

<210> SEQ ID NO 29
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-10 Alanine scan F-11-A-11

<400> SEQUENCE: 29

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Ala Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 30
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-11 Alanine scan D-12-A-12

<400> SEQUENCE: 30

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Ala Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 31
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-12srmb scrambled version

<400> SEQUENCE: 31

Arg Arg Arg Tyr Val Tyr Asn Gly Trp Gly Tyr Phe Glu Gly Ala Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 32
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-22 Glutamic acid scan G-2-E-2

<400> SEQUENCE: 32

Arg Arg Arg Glu Glu Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 33
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-23 Glutamic acid scan G-3-E-3

<400> SEQUENCE: 33

Arg Arg Arg Glu Gly Glu Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

-continued

<210> SEQ ID NO 34
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-24 Glutamic acid scan G-4-E-4

<400> SEQUENCE: 34

Arg Arg Arg Glu Gly Gly Glu Tyr Tyr Val Asn Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 35
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-26 Glutamic acid scan Y-5-E-5

<400> SEQUENCE: 35

Arg Arg Arg Glu Gly Gly Gly Glu Tyr Val Asn Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 36
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-27 Glutamic acid scan Y-6-E-6

<400> SEQUENCE: 36

Arg Arg Arg Glu Gly Gly Gly Tyr Glu Val Asn Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 37
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-28 Glutamic acid scan V-7-E-7

<400> SEQUENCE: 37

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Glu Asn Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 38
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-29 Glutamic acid scan N-8-E-8

<400> SEQUENCE: 38

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Glu Trp Tyr Phe Asp Arg
1 5 10 15

-continued

Arg Arg

<210> SEQ ID NO 39
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-30 Glutamic acid scan W-9-E-9

<400> SEQUENCE: 39

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Glu Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 40
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-31 Glutamic acid scan Y-10-E-10

<400> SEQUENCE: 40

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Asn Trp Glu Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 41
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-32 Glutamic acid scan F-11-E-11

<400> SEQUENCE: 41

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Glu Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 42
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-33 Glutamic acid scan D12-E-12

<400> SEQUENCE: 42

Arg Arg Arg Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Glu Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 43
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/A1 derived mutant peptide A1/3-34srmb scrambled version

<400> SEQUENCE: 43

Arg Arg Arg Gly Glu Tyr Gly Glu Tyr Trp Asn Gly Asp Phe Tyr Arg
1 5 10 15

-continued

Arg Arg

<210> SEQ ID NO 44

<400> SEQUENCE: 44

000

<210> SEQ ID NO 45
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/B1 derived mutated peptide B1/4

<400> SEQUENCE: 45

Arg Glu Gly Gly Gly Phe Thr Val Asn Trp Tyr Phe Asp Arg
1 5 10

<210> SEQ ID NO 46
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/B1 derived mutated peptide B1/5 scrambled version

<400> SEQUENCE: 46

Phe Gly Val Gly Tyr Arg Gly Glu Thr Arg Asn Phe Asp Trp
1 5 10

<210> SEQ ID NO 47
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/B1 derived mutated peptide B1/6

<400> SEQUENCE: 47

Glu Glu Glu Glu Gly Gly Gly Phe Thr Val Asn Trp Tyr Phe Asp Glu
1 5 10 15

Glu Glu

<210> SEQ ID NO 48
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/B1 derived mutated peptide B1/7

<400> SEQUENCE: 48

Arg Arg Arg Glu Gly Gly Gly Phe Thr Val Asn Trp Tyr Phe Asp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 49
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:antibody 198/B1 derived mutated peptide B1/7scr3 scrambled version

-continued

<400> SEQUENCE: 49

Arg Arg Arg Phe Gly Val Gly Tyr Gly Glu Thr Asn Phe Asp Trp Arg
1 5 10 15

Arg Arg

<210> SEQ ID NO 50
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H back primer VH1BACK-SfiI

<400> SEQUENCE: 50

catgccatga ctcgcggccc agccggccat ggccsaggts marctgcags agtcwgg 57

<210> SEQ ID NO 51
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H back primer VH1BACKSfi

<400> SEQUENCE: 51

gtcctcgcaa ctgcggccca gccggccatg gccgaggtgc agcttcagga gtcagg 56

<210> SEQ ID NO 52
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H back primer VH2BACKSfi

<400> SEQUENCE: 52

gtcctcgcaa ctgcggccca gccggccatg gccgatgtgc agcttcagga gtcrgg 56

<210> SEQ ID NO 53
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H back primer VH3BACKSfi

<400> SEQUENCE: 53

gtcctcgcaa ctgcggccca gccggccatg gcccaggtgc agctgaagsa gtcagg 56

<210> SEQ ID NO 54
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H back primer VH4/6BACKSfi

<400> SEQUENCE: 54

gtcctcgcaa ctgcggccca gccggccatg gccgaggtyc agctgcarca rtctgg 56

<210> SEQ ID NO 55
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H

-continued

back primer VH5/9BACKSfi

<400> SEQUENCE: 55

gtcctcgcaa ctgcggccca gccggccatg gcccaggtyc arctgcagca gyctgg 56

<210> SEQ ID NO 56
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H back primer VH7BACKSfi

<400> SEQUENCE: 56

gtcctcgcaa ctgcggccca gccggccatg gccgargtga agctggtgga rtctgg 56

<210> SEQ ID NO 57
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H back primer VH8BACKSfi

<400> SEQUENCE: 57

gtcctcgcaa ctgcggccca gccggccatg gccgaggttc agcttcagca gtctgg 56

<210> SEQ ID NO 58
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H back primer VH10BACKSfi

<400> SEQUENCE: 58

gtcctcgcaa ctgcggccca gccggccatg gccgaagtgc agctgktgga gwctgg 56

<210> SEQ ID NO 59
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-H back primer VH11BACKSfi

<400> SEQUENCE: 59

gtcctcgcaa ctgcggccca gccggccatg gcccagatcc agttgctgca gtctgg 56

<210> SEQ ID NO 60
<211> LENGTH: 68
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse J-H forward primer VH1FOR2LiAsc

<400> SEQUENCE: 60

accgccagag gcgcgcccac ctgaaccgcc tccacctgag gagacggtga ccgtggtccc 60

ttggcccc 68

<210> SEQ ID NO 61
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Description of Artificial Sequence:mouse J-H forward primer JH1FORLiAsc

<400> SEQUENCE: 61

accgccagag gcgcgcccac ctgaaccgcc tccacctgag gagacggtga ccgtggtccc 60

<210> SEQ ID NO 62
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse J-H forward primer JH2FORLiAsc

<400> SEQUENCE: 62

accgccagag gcgcgcccac ctgaaccgcc tccacctgag gagactgtga gagtggtgcc 60

<210> SEQ ID NO 63
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse J-H forward primer JH3FORLiAsc

<400> SEQUENCE: 63

accgccagag gcgcgcccac ctgaaccgcc tccacctgca gagacagtga ccagagtccc 60

<210> SEQ ID NO 64
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse J-H forward primer JH4FORLiAsc

<400> SEQUENCE: 64

accgccagag gcgcgcccac ctgaaccgcc tccacctgag gagacggtga ctgaggttcc 60

<210> SEQ ID NO 65
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-kappa back primer VK2BACK-LiAscI

<400> SEQUENCE: 65

ggttcagatg ggcgcgcctc tggcggtggc ggatcggaca ttgagctcac ccagtctcca 60

<210> SEQ ID NO 66
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse V-kappa back primer VK1BACKLi Asc

<400> SEQUENCE: 66

ggttcagatg ggcgcgcctc tggcggtggc ggatcggaca ttgtgatgwc acagtctcc 59

<210> SEQ ID NO 67
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse

-continued

V-kappa back primer VK2BACKLi Asc

<400> SEQUENCE: 67

ggttcagatg ggcgcgcctc tggcggtggc ggatcggatg ttktgatgac ccaaactcc 59

<210> SEQ ID NO 68
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse
V-kappa back primer VK3BACKLi Asc

<400> SEQUENCE: 68

ggttcagatg ggcgcgcctc tggcggtggc ggatcggata ttgtgatrac bcaggcwgc 59

<210> SEQ ID NO 69
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse
V-kappa back primer VK4BACKLi Asc

<400> SEQUENCE: 69

ggttcagatg ggcgcgcctc tggcggtggc ggatcggaca ttgtgctgac mcartctcc 59

<210> SEQ ID NO 70
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse
V-kappa back primer VK5BACKLi Asc

<400> SEQUENCE: 70

ggttcagatg ggcgcgcctc tggcggtggc ggatcgsaaa wtgtkctcac ccagtctcc 59

<210> SEQ ID NO 71
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse
V-kappa back primer VK6BACKLi Asc

<400> SEQUENCE: 71

ggttcagatg ggcgcgcctc tggcggtggc ggatcggaya tyvwgatgac mcagwctcc 59

<210> SEQ ID NO 72
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse
V-kappa back primer VK7BACKLi Asc

<400> SEQUENCE: 72

ggttcagatg ggcgcgcctc tggcggtggc ggatcgcaaa ttgttctcac ccagtctcc 59

<210> SEQ ID NO 73
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse
V-kappa back primer VK8BACKLi Asc

-continued

<400> SEQUENCE: 73

ggttcagatg ggcgcgcctc tggcggtggc ggatcgtcat tattgcaggt gcttgtggg 59

<210> SEQ ID NO 74
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse J-kappa forward primer JK1NOT10

<400> SEQUENCE: 74

gagtcattct gcggccgccc gtttgatttc cagcttggtg cc 42

<210> SEQ ID NO 75
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse J-kappa forward primer JK2NOT10

<400> SEQUENCE: 75

gagtcattct gcggccgccc gttttatttc cagcttggtc cc 42

<210> SEQ ID NO 76
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse J-kappa forward primer JK3NOT10

<400> SEQUENCE: 76

gagtcattct gcggccgccc gttttatttc agtctggtc cc 42

<210> SEQ ID NO 77
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse J-kappa forward primer JK4NOT10

<400> SEQUENCE: 77

gagtcattct gcggccgccc gttttatttc caactttgtc cc 42

<210> SEQ ID NO 78
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mouse J-kappa forward primer JK5NOT10

<400> SEQUENCE: 78

gagtcattct gcggccgccc gtttcagctc cagcttggtc cc 42

<210> SEQ ID NO 79
<211> LENGTH: 74
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: oligonucleotide mychis6-co

-continued

<400> SEQUENCE: 79

ggccgcagaa caaaaactca tctcagaaga ggatctgaat ggggcggcac atcaccatca 60

ccatcactaa taag 74

<210> SEQ ID NO 80
<211> LENGTH: 74
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
oligonucleotide mycchis-ic

<400> SEQUENCE: 80

aattcttatt agtgatggtg atggtgatgt gccgccccat tcagatcctc ttctgagatg 60

agtttttgtt ctgc 74

<210> SEQ ID NO 81
<211> LENGTH: 726
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:scFv from
hybridoma cell line 193/AD3

<400> SEQUENCE: 81

gaggtgaagc tggtggagtc tggacctgag ctgaagaagc ctggagagac agtcaagatc 60

tcctgcaagg cttctgggta tatcttcaca aactatggaa tgaactgggt gaagcaggct 120

ccaggaaagg gtttaaagtg gatgggctgg ataaacacct acactggaga gccaacatat 180

gctgatgact tcaagggacg gtttgccttc tctttggaaa cctctgccag cactgcctat 240

ttgcagatca acaacctcaa aaatgaggac acggctacat atttctgtgc attatatggt 300

aactccccta aggggtttgc ttactggggc caagggactc tggtcactgt ctctgcaggt 360

ggaggcggtt caggtgggcg cgcctctggc ggtggcggat cggatattca gatgacacag 420

tctcccaaat tcctgcttgt atcagcagga gacagggtta ccataacctg caaggccagt 480

cagagtgtga gtaatgatgt agcttggtac caacagaagc cggggcagtc tcctaaacta 540

ctgatgtact atgcatccaa tcgctacact ggagtccctg atcgcttcac tggcagtgga 600

tatgggacgg atttcacttt caccatcagc actgtgcagg ctgaagacct ggcagtttat 660

ttctgtcagc aggattatgg ctctcctccc acgttcggag ggggcaccaa gctggaaatt 720

aaacgg 726

<210> SEQ ID NO 82
<211> LENGTH: 242
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:scFv from
hybridoma cell line 193/AD3

<400> SEQUENCE: 82

Glu Val Lys Leu Val Glu Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ile Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe

-continued

```
     50                  55                  60

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
 65                  70                  75                  80

Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
                85                  90                  95

Ala Leu Tyr Gly Asn Ser Pro Lys Gly Phe Ala Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ala Gly Gly Gly Gly Ser Gly Gly Arg Ala
        115                 120                 125

Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Lys Phe
    130                 135                 140

Leu Leu Val Ser Ala Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser
145                 150                 155                 160

Gln Ser Val Ser Asn Asp Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln
                165                 170                 175

Ser Pro Lys Leu Leu Met Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val
            180                 185                 190

Pro Asp Arg Phe Thr Gly Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr
        195                 200                 205

Ile Ser Thr Val Gln Ala Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln
    210                 215                 220

Asp Tyr Gly Ser Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
225                 230                 235                 240

Lys Arg
```

<210> SEQ ID NO 83
<211> LENGTH: 747
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:scFv from hybridoma cell line 193/K2

<400> SEQUENCE: 83

```
gaagtgcagc tggtggagtc tgggggaggc ctagtgaagc ctggagggtc cctgaaactc     60

tcctgtgcag cctctggatt cactttcagt acctatacca tgtcttgggt tcgccagact    120

ccggagaaga ggctggagtg ggtcgcaacc attagtagtg gtggtagtta cacctactat    180

ccagacagtg tgaggggccg attcaccatc tccagagaca atgccaagaa caccctgtac    240

ctgcaaatga gcagtctgaa gtctgaggac acagccatgt attactgtac aagagatggg    300

ggacacgggt acggtagtag ctttgactac tggggccaag gcaccactct cacagtctcc    360

tcaggtggag gcggttcagg tgggcgcgcc tctggcggtg gcggatcgca aattgtgctc    420

acccagtctc cactctccct gcctgtcagt cttggagatc aagcctccat ctcttgcaga    480

tctagtcaga gcattgtaca tagtaatgga aacacctatt tagaatggta cctgcagaaa    540

ccaggccagt ctccaaagct cctgatctac aaagtttcca accgattttc tggggtccca    600

gacaaattca gtggcagtgg atcagggaca gatttcacac tcaagatcag cagagtggag    660

gctgaggatc tgggagttta ttactgcttt caaggttcac atgttccgtg gacgttcggt    720

ggaggcacca agctggaaat caaacgg                                        747
```

<210> SEQ ID NO 84
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:scFv from hybridoma cell line 193/K2

<400> SEQUENCE: 84

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
20 25 30

Thr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

Ala Thr Ile Ser Ser Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Arg Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Thr Arg Asp Gly Gly His Gly Tyr Gly Ser Ser Phe Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Arg Ala Ser Gly Gly Gly Gly Ser Gln Ile Val Leu Thr Gln Ser Pro
130 135 140

Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg
145 150 155 160

Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp
165 170 175

Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val
180 185 190

Ser Asn Arg Phe Ser Gly Val Pro Asp Lys Phe Ser Gly Ser Gly Ser
195 200 205

Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu
210 215 220

Gly Val Tyr Tyr Cys Phe Gln Gly Ser His Val Pro Trp Thr Phe Gly
225 230 235 240

Gly Gly Thr Lys Leu Glu Ile Lys Arg
245

<210> SEQ ID NO 85
<211> LENGTH: 747
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:scFv from hybridoma cell line 198/AB2 (subclone of 198/B1)

<400> SEQUENCE: 85

gaggtgcagc ttcaggagtc aggggaggc ttagtgaagc ctggagggtc cctgaaactc 60

tcctgtgcag cctctggatt cactttcagt agctatacca tgtcttgggt tcgccagact 120

ccggagaaga ggctggagtg ggtcgcaacc attagtagtg gtggtagttc cacctactat 180

ccagacagtg tgaagggccg attcaccatc tccagagaca atgccaagaa caccctgtac 240

ctgcaaatga gcagtctgag gtctgaggac acagccatgt attactgtac aagagagggg 300

ggtggtttca ccgtcaactg gtacttcgat gtctggggcg cagggactct ggtcactgtc 360

tctgcaggtg gaggcggttc aggtgggcgc gcctctggcg gtggcggatc ggaaaatgtg 420

-continued

ctcacccagt ctccagcttc tttggctgtg tctctagggc agagggccac catatcctgc 480

agagccagtg aaagtgttga tagttatggc tataatttta tgcactggta tcagcagata 540

ccaggacagc cacccaaact cctcatctat cgtgcatcca acctagagtc tgggatccct 600

gccaggttca gtggcagtgg gtctaggaca gacttcaccc tcaccattaa tcctgtggag 660

gctgatgatg ttgcaaccta ttactgtcag caaagtaatg aggatccgct cacgttcggt 720

actgggacca gactggaaat aaaacgg 747

<210> SEQ ID NO 86
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:scFv from hybridoma cell line 198/AB2 (subclone of 198/B1)

<400> SEQUENCE: 86

Glu Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Thr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

Ala Thr Ile Ser Ser Gly Gly Ser Ser Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Thr Arg Glu Gly Gly Gly Phe Thr Val Asn Trp Tyr Phe Asp Val Trp
100 105 110

Gly Ala Gly Thr Leu Val Thr Val Ser Ala Gly Gly Gly Gly Ser Gly
115 120 125

Gly Arg Ala Ser Gly Gly Gly Gly Ser Glu Asn Val Leu Thr Gln Ser
130 135 140

Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys
145 150 155 160

Arg Ala Ser Glu Ser Val Asp Ser Tyr Gly Tyr Asn Phe Met His Trp
165 170 175

Tyr Gln Gln Ile Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Arg Ala
180 185 190

Ser Asn Leu Glu Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser
195 200 205

Arg Thr Asp Phe Thr Leu Thr Ile Asn Pro Val Glu Ala Asp Asp Val
210 215 220

Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Glu Asp Pro Leu Thr Phe Gly
225 230 235 240

Thr Gly Thr Arg Leu Glu Ile Lys Arg
245

<210> SEQ ID NO 87
<211> LENGTH: 747
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:scFv derived from hybridima cell line 198/A1

-continued

<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(747)
<223> OTHER INFORMATION: n = g, a, c or t

<400> SEQUENCE: 87

gaggtgcagc ttcaggagtc aggggaggc ttagtgaagc ctggagggtc cctgaaactc 60

tcctgtgcag cctctggatt cattttagt agttatacca tgtcttgggt tcgccagact 120

ccggagaaga ggctggagtg ggtcgcaacc attagtagtg gtggtagttc cacctactat 180

ccagacagtg tgaagggccg attcaccatc tccagagaca atgccaagaa caccctgtac 240

ctgcaaatga gcagtctgaa gtctgaggac acagccatgt atcactgtac aagagagggg 300

ggtggttatt acgtcaactg gtacttcgat gtctggggcg caggcaccac tctcacagtc 360

tcctcaggtg gaggcggttc aggtgggcgc gcctctggcg gtggcggatc ggacattgag 420

ctcacncagt ctccagcttc tttggctgtg tctctagggc agagggccac catatcctgc 480

agagccagtg aaagtgttga tagttatggc aagagtttta tgcactggta ccagcagaaa 540

ccagggcagc cacccaaact cctcatctat cgtgcatcca acctagaatc tgggatccct 600

gccaggttca gtggcagtgg gtctaggaca gacttcaccc tcaccattaa tcctgtggag 660

gctgatgatg ttgcnaccta ttactgtcag caaagtaatg aggatcccct cacgttcggt 720

gctgggacca gactggaaat aaaacgg 747

<210> SEQ ID NO 88
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:scFv derived from hybridima cell line 198/A1

<400> SEQUENCE: 88

Glu Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Ser Ser Tyr
20 25 30

Thr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

Ala Thr Ile Ser Ser Gly Gly Ser Ser Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr His Cys
85 90 95

Thr Arg Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp Val Trp
100 105 110

Gly Ala Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
115 120 125

Gly Arg Ala Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser
130 135 140

Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys
145 150 155 160

Arg Ala Ser Glu Ser Val Asp Ser Tyr Gly Lys Ser Phe Met His Trp
165 170 175

Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Arg Ala
180 185 190

-continued

```
Ser Asn Leu Glu Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser
        195                 200                 205

Arg Thr Asp Phe Thr Leu Thr Ile Asn Pro Val Glu Ala Asp Asp Val
    210                 215                 220

Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Glu Asp Pro Leu Thr Phe Gly
225                 230                 235                 240

Ala Gly Thr Arg Leu Glu Ile Lys Arg
                245
```

<210> SEQ ID NO 89
<211> LENGTH: 2199
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:198A2 scFv-alkaline phosphatase fusion protein (ORF of expression vector pDAP2-198AB2#100)
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (228)
<223> OTHER INFORMATION: n = g, a, c or t

<400> SEQUENCE: 89

```
atgaaatacc tattgcctac ggcagccgct ggattgttat tactcgcggc ccagccggcc     60

atggcggagg tgaagctggt ggagtctggg ggaggcttag tgaagcctgg agggtccctg    120

aaactctcct gtgcagcctc tggattcact ttcagtagct ataccatgtc ttgggttcgc    180

cagactccgg agaagaggct ggagtgggtc gcaaccatta gtagtggngg tagttccacc    240

tactatccag acagtgtgaa gggccgattc accatctcca gagacaatgc caagaacacc    300

ctgtacctgc aaatgagcag tctgaggtct gaggacacag ccatgtatta ctgtacaaga    360

gaggggggtg gtttcaccgt caactggtac ttcgatgtct ggggcgcagg aacctcagtc    420

accgtctcct caggtggagg cggttcaggt gggcgcgcct ctggcggtgg cggatcggac    480

attgtgctga cacagtctcc agcttctttg gctgtgtctc tagggcagag ggccaccata    540

tcctgcagag ccagtgaaag tgttgatagt tatggctata attttatgca ctggtatcag    600

cagataccag gacagccacc caaactcctc atctatcgtg catccaacct agagtctggg    660

atccctgcca ggttcagtgg cagtgggtct aggacagact tcaccctcac cattaatcct    720

gtggaggctg atgatgttgc aacctattac tgtcagcaaa gtaatgagga tccgctcacg    780

ttcggtactg ggaccagact ggaaataaaa cgggcggccg cagcccgggc accagaaatg    840

cctgttctgg aaaaccgggc tgctcagggc gatattactg cacccggcgg tgctcgccgt    900

ttaacgggtg atcagactgc cgctctgcgt gattctctta gcgataaacc tgcaaaaaat    960

attattttgc tgattggcga tgggatgggg gactcggaaa ttactgccgc acgtaattat   1020

gccgaaggtg cgggcggctt ttttaaaggt atagatgcct taccgcttac cgggcaatac   1080

actcactatg cgctgaataa aaaaaccggc aaaccggact acgtcaccga ctcggctgca   1140

tcagcaaccg cctggtcaac cggtgtcaaa acctataacg gcgcgctggg cgtcgatatt   1200

cacgaaaaag atcacccaac gattctggaa atggcaaaag ccgcaggtct ggcgaccggt   1260

aacgtttcta ccgcagagtt gcaggatgcc acgcccgctg cgctggtggc acatgtgacc   1320

tcgcgcaaat gctacggtcc gagcgcgacc agtgaaaaat gtccgggtaa cgctctggaa   1380

aaaggcggaa aaggatcgat taccgaacag ctgcttaacg ctcgtgccga cgttacgctt   1440

ggcggcggcg caaaaacctt tgctgaaacg gcaaccgctg gtgaatggca gggaaaaacg   1500
```

-continued

```
ctgcgtgaac aggcacaggc gcgtggttat cagttggtga gcgatgctgc ctcactgaat   1560

tcggtgacgg aagcgaatca gcaaaaaccc ctgcttggcc tgtttgctga cggcaatatg   1620

ccagtgcgct ggctaggacc gaaagcaacg taccatggca atatcgataa gcccgcagtc   1680

acctgtacgc caaatccgca acgtaatgac agtgtaccaa ccctggcgca gatgaccgac   1740

aaagccattg aattgttgag taaaaatgag aaaggctttt tcctgcaagt tgaaggtgcg   1800

tcaatcgata aacaggatca tgctgcgaat ccttgtgggc aaattggcga gacggtcgat   1860

ctcgatgaag ccgtacaacg ggcgctggaa ttcgctaaaa aggagggtaa cacgctggtc   1920

atagtcaccg ctgatcacgc ccacgccagc cagattgttg cgccggatac caaagctccg   1980

ggcctcaccc aggcgctaaa taccaaagat ggcgcagtga tggtgatgag ttacgggaac   2040

tccgaagagg attcacaaga acataccggc agtcagttgc gtattgcggc gtatggcccg   2100

catgccgcca atgttgttgg actgaccgac cagaccgatc tcttctacac catgaaagcc   2160

gctctggggg atatcgcaca ccatcaccat caccattaa                          2199
```

<210> SEQ ID NO 90
<211> LENGTH: 732
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:198A2 scFv-alkaline phosphatase fusion protein (ORF of expression vector pDAP2-198AB2#100)

<400> SEQUENCE: 90

```
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
  1               5                  10                  15

Ala Gln Pro Ala Met Ala Glu Val Lys Leu Val Glu Ser Gly Gly Gly
             20                  25                  30

Leu Val Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly
         35                  40                  45

Phe Thr Phe Ser Ser Tyr Thr Met Ser Trp Val Arg Gln Thr Pro Glu
     50                  55                  60

Lys Arg Leu Glu Trp Val Ala Thr Ile Ser Ser Gly Gly Ser Ser Thr
 65                  70                  75                  80

Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
                 85                  90                  95

Ala Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Arg Ser Glu Asp
            100                 105                 110

Thr Ala Met Tyr Tyr Cys Thr Arg Glu Gly Gly Gly Phe Thr Val Asn
        115                 120                 125

Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr Ser Val Thr Val Ser Ser
    130                 135                 140

Gly Gly Gly Gly Ser Gly Gly Arg Ala Ser Gly Gly Gly Gly Ser Asp
145                 150                 155                 160

Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln
                165                 170                 175

Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Ser Tyr Gly
            180                 185                 190

Tyr Asn Phe Met His Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro Lys
        195                 200                 205

Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Ile Pro Ala Arg
    210                 215                 220

Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Asn Pro
```

-continued

```
225                 230                 235                 240

Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Glu
                245                 250                 255

Asp Pro Leu Thr Phe Gly Thr Gly Thr Arg Leu Glu Ile Lys Arg Ala
            260                 265                 270

Ala Ala Ala Arg Ala Pro Glu Met Pro Val Leu Glu Asn Arg Ala Ala
        275                 280                 285

Gln Gly Asp Ile Thr Ala Pro Gly Gly Ala Arg Arg Leu Thr Gly Asp
    290                 295                 300

Gln Thr Ala Ala Leu Arg Asp Ser Leu Ser Asp Lys Pro Ala Lys Asn
305                 310                 315                 320

Ile Ile Leu Leu Ile Gly Asp Gly Met Gly Asp Ser Glu Ile Thr Ala
                325                 330                 335

Ala Arg Asn Tyr Ala Glu Gly Ala Gly Gly Phe Phe Lys Gly Ile Asp
            340                 345                 350

Ala Leu Pro Leu Thr Gly Gln Tyr Thr His Tyr Ala Leu Asn Lys Lys
        355                 360                 365

Thr Gly Lys Pro Asp Tyr Val Thr Asp Ser Ala Ala Ser Ala Thr Ala
    370                 375                 380

Trp Ser Thr Gly Val Lys Thr Tyr Asn Gly Ala Leu Gly Val Asp Ile
385                 390                 395                 400

His Glu Lys Asp His Pro Thr Ile Leu Glu Met Ala Lys Ala Ala Gly
                405                 410                 415

Leu Ala Thr Gly Asn Val Ser Thr Ala Glu Leu Gln Asp Ala Thr Pro
            420                 425                 430

Ala Ala Leu Val Ala His Val Thr Ser Arg Lys Cys Tyr Gly Pro Ser
        435                 440                 445

Ala Thr Ser Glu Lys Cys Pro Gly Asn Ala Leu Glu Lys Gly Gly Lys
    450                 455                 460

Gly Ser Ile Thr Glu Gln Leu Leu Asn Ala Arg Ala Asp Val Thr Leu
465                 470                 475                 480

Gly Gly Gly Ala Lys Thr Phe Ala Glu Thr Ala Thr Ala Gly Glu Trp
                485                 490                 495

Gln Gly Lys Thr Leu Arg Glu Gln Ala Gln Ala Arg Gly Tyr Gln Leu
            500                 505                 510

Val Ser Asp Ala Ala Ser Leu Asn Ser Val Thr Glu Ala Asn Gln Gln
        515                 520                 525

Lys Pro Leu Leu Gly Leu Phe Ala Asp Gly Asn Met Pro Val Arg Trp
    530                 535                 540

Leu Gly Pro Lys Ala Thr Tyr His Gly Asn Ile Asp Lys Pro Ala Val
545                 550                 555                 560

Thr Cys Thr Pro Asn Pro Gln Arg Asn Asp Ser Val Pro Thr Leu Ala
                565                 570                 575

Gln Met Thr Asp Lys Ala Ile Glu Leu Leu Ser Lys Asn Glu Lys Gly
            580                 585                 590

Phe Phe Leu Gln Val Glu Gly Ala Ser Ile Asp Lys Gln Asp His Ala
        595                 600                 605

Ala Asn Pro Cys Gly Gln Ile Gly Glu Thr Val Asp Leu Asp Glu Ala
    610                 615                 620

Val Gln Arg Ala Leu Glu Phe Ala Lys Lys Glu Gly Asn Thr Leu Val
625                 630                 635                 640

Ile Val Thr Ala Asp His Ala His Ala Ser Gln Ile Val Ala Pro Asp
                645                 650                 655
```

-continued

```
Thr Lys Ala Pro Gly Leu Thr Gln Ala Leu Asn Thr Lys Asp Gly Ala
            660                 665                 670

Val Met Val Met Ser Tyr Gly Asn Ser Glu Glu Asp Ser Gln Glu His
        675                 680                 685

Thr Gly Ser Gln Leu Arg Ile Ala Ala Tyr Gly Pro His Ala Ala Asn
    690                 695                 700

Val Val Gly Leu Thr Asp Gln Thr Asp Leu Phe Tyr Thr Met Lys Ala
705                 710                 715                 720

Ala Leu Gly Asp Ile Ala His His His His His His
                725                 730
```

<210> SEQ ID NO 91
<211> LENGTH: 978
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:plasmid pZip-198AB2#102
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(978)
<223> OTHER INFORMATION: n = g, a, c or t

<400> SEQUENCE: 91

```
atgaaatacc tattgcctac ggcagccgct ggattgttat tactcgcggc ccagccggcc     60

atggcggagg tgaagctggt ggagtctggg ggaggcttag tgaagcctgg agggtccctg    120

aaactctcct gtgcagcctc tggattcact ttcagtagct ataccatgtc ttgggttcgc    180

cagactccgg agaagaggct ggagtgggtc gcaaccatta gtagtggngg tagttccacc    240

tactatccag acagtgtgaa gggccgattc accatctcca gagacaatgc caagaacacc    300

ctgtacctgc aaatgagcag tctgaggtct gaggacacag ccatgtatta ctgtacaaga    360

gagggggtg gtttcaccgt caactggtac ttcgatgtct ggggcgcagg aacctcagtc    420

accgtctcct caggtggagg cggttcaggt gggcgcgcct ctggcggtgg cggatcggac    480

attgtgctga cacagtntcc agcttctttg gctgtgtctc tagggcagag ggccaccata    540

tcntgcagag ccagtgaaag tgttgatagt tatggctata attttatgca ctggtatcag    600

cagataccag gacagccacc caaactcctc atctatcgtg catccaacct agagtctggg    660

atccctgcca ggttcagtgg cagtgggtct aggacagact tcaccctcac cattaatcct    720

gtggaggctg atgatgttgc aacctattac tgtcagcaaa gtaatgagga tccgctcacg    780

ttcggtactg ggaccagact ggaaataaaa cgggcggccg caccgaagcc ttccactccg    840

cccgggtctt cccgtatgaa acagctggaa gacaaagtag aggagctcct tagcaagaac    900

taccatctag aaaacgaggt agctcgtctg aaaaagcttg ttggtgaacg tggtggtcac    960

catcaccatc accattaa                                                  978
```

<210> SEQ ID NO 92
<211> LENGTH: 325
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:plasmid pZip-198AB2#102
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (166)
<223> OTHER INFORMATION: Xaa = Cys, Tyr, Ser or Phe

<400> SEQUENCE: 92

-continued

```
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
  1               5                  10                  15

Ala Gln Pro Ala Met Ala Glu Val Lys Leu Val Glu Ser Gly Gly Gly
             20                  25                  30

Leu Val Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly
         35                  40                  45

Phe Thr Phe Ser Ser Tyr Thr Met Ser Trp Val Arg Gln Thr Pro Glu
     50                  55                  60

Lys Arg Leu Glu Trp Val Ala Thr Ile Ser Ser Gly Gly Ser Ser Thr
 65                  70                  75                  80

Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
                 85                  90                  95

Ala Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Arg Ser Glu Asp
            100                 105                 110

Thr Ala Met Tyr Tyr Cys Thr Arg Glu Gly Gly Gly Phe Thr Val Asn
        115                 120                 125

Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr Ser Val Thr Val Ser Ser
    130                 135                 140

Gly Gly Gly Gly Ser Gly Gly Arg Ala Ser Gly Gly Gly Gly Ser Asp
145                 150                 155                 160

Ile Val Leu Thr Gln Xaa Pro Ala Ser Leu Ala Val Ser Leu Gly Gln
                165                 170                 175

Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Ser Tyr Gly
            180                 185                 190

Tyr Asn Phe Met His Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro Lys
        195                 200                 205

Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Ile Pro Ala Arg
    210                 215                 220

Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Asn Pro
225                 230                 235                 240

Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Glu
                245                 250                 255

Asp Pro Leu Thr Phe Gly Thr Gly Thr Arg Leu Glu Ile Lys Arg Ala
            260                 265                 270

Ala Ala Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Arg Met Lys Gln
        275                 280                 285

Leu Glu Asp Lys Val Glu Glu Leu Leu Ser Lys Asn Tyr His Leu Glu
    290                 295                 300

Asn Glu Val Ala Arg Leu Lys Lys Leu Val Gly Glu Arg Gly Gly His
305                 310                 315                 320

His His His His His
                325
```

<210> SEQ ID NO 93
<211> LENGTH: 2190
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mAB#8860 scFv-alkaline phosphatase fusion protein (vector construct pDAP2-8860scFv#11)

<400> SEQUENCE: 93

```
atgaaatacc tattgcctac ggcagccgct ggattgttat tactcgcggc ccagccggcc     60

atggccgagg ttcagcttca gcagtctgga cctgagctgg tgaagcccgg ggcctcagtg    120
```

-continued

aagatttcct gcaaagcttc tggctacgca ttcagtagct cttggatgaa ctgggtgaag 180

cagaggcctg gacagggtct tgagtggatt ggacggattt atcctggaaa tggagatact 240

aactacaatg ggaagttcaa gggcaaggcc acactgactg cagacaaatc ctccagcaca 300

gcctacatgc agctcagcag cctgacctct gtggactctg cggtctattt ctgtgcagat 360

ggtaacgtat attactatgc tatggactac tggggtcaag gaacctcagt caccgtctcc 420

tcaggtggag gcggttcagg tgggcgcgcc tctggcggtg gcggatcgca aattgttctc 480

acccagtctc ctgcttcctt agctgtatct ctggggcaga gggccaccat ctcatgcagg 540

gccagcaaaa gtgtcagtac atctggctat agttatatgc actggtacca acagaaacca 600

ggacagccac ccaaactcct catctatctt gcatccaacc tagaatctgg ggtccctgcc 660

aggttcagtg gcagtgggtc tgggacagac ttcaccctca acatccatcc tgtggaggag 720

gaggatgctg caacctatta ctgtcagcac agtagggagc ttcctcggac gttcggtgga 780

ggcaccaagc tggaaatcaa acgggcggcc gcagcccggg caccagaaat gcctgttctg 840

gaaaaccggg ctgctcaggg cgatattact gcacccggcg gtgctcgccg tttaacgggt 900

gatcagactg ccgctctgcg tgattctctt agcgataaac ctgcaaaaaa tattattttg 960

ctgattggcg atgggatggg ggactcggaa attactgccg cacgtaatta tgccgaaggt 1020

gcgggcggct tttttaaagg tatagatgcc ttaccgctta ccgggcaata cactcactat 1080

gcgctgaata aaaaaaccgg caaaccggac tacgtcaccg actcggctgc atcagcaacc 1140

gcctggtcaa ccggtgtcaa aacctataac ggcgcgctgg gcgtcgatat tcacgaaaaa 1200

gatcacccaa cgattctgga aatggcaaaa gccgcaggtc tggcgaccgg taacgtttct 1260

accgcagagt tgcaggatgc cacgcccgct gcgctggtgg cacatgtgac ctcgcgcaaa 1320

tgctacggtc cgagcgcgac cagtgaaaaa tgtccgggta acgctctgga aaaaggcgga 1380

aaaggatcga ttaccgaaca gctgcttaac gctcgtgccg acgttacgct tggcggcggc 1440

gcaaaaacct ttgctgaaac ggcaaccgct ggtgaatggc agggaaaaac gctgcgtgaa 1500

caggcacagg cgcgtggtta tcagttggtg agcgatgctg cctcactgaa ttcggtgacg 1560

gaagcgaatc agcaaaaacc cctgcttggc ctgtttgctg acggcaatat gccagtgcgc 1620

tggctaggac cgaaagcaac gtaccatggc aatatcgata agcccgcagt cacctgtacg 1680

ccaaatccgc aacgtaatga cagtgtacca accctggcgc agatgaccga caaagccatt 1740

gaattgttga gtaaaaatga gaaaggcttt ttcctgcaag ttgaaggtgc gtcaatcgat 1800

aaacaggatc atgctgcgaa tccttgtggg caaattggcg agacggtcga tctcgatgaa 1860

gccgtacaac gggcgctgga attcgctaaa aaggagggta acacgctggt catagtcacc 1920

gctgatcacg cccacgccag ccagattgtt gcgccggata ccaaagctcc gggcctcacc 1980

caggcgctaa ataccaaaga tggcgcagtg atggtgatga gttacgggaa ctccgaagag 2040

gattcacaag aacataccgg cagtcagttg cgtattgcgg cgtatggccc gcatgccgcc 2100

aatgttgttg gactgaccga ccagaccgat ctcttctaca ccatgaaagc cgctctgggg 2160

gatatcgcac accatcacca tcaccattaa 2190

<210> SEQ ID NO 94
<211> LENGTH: 729
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mAB#8860 scFv-alkaline phosphatase fusion protein (vector construct

-continued

pDAP2-8860scFv#11)

<400> SEQUENCE: 94

```
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
  1               5                  10                  15

Ala Gln Pro Ala Met Ala Glu Val Gln Leu Gln Gln Ser Gly Pro Glu
             20                  25                  30

Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly
         35                  40                  45

Tyr Ala Phe Ser Ser Ser Trp Met Asn Trp Val Lys Gln Arg Pro Gly
     50                  55                  60

Gln Gly Leu Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asn Gly Asp Thr
 65                  70                  75                  80

Asn Tyr Asn Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys
                 85                  90                  95

Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Val Asp
            100                 105                 110

Ser Ala Val Tyr Phe Cys Ala Asp Gly Asn Val Tyr Tyr Tyr Ala Met
        115                 120                 125

Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly
    130                 135                 140

Gly Ser Gly Gly Arg Ala Ser Gly Gly Gly Gly Ser Gln Ile Val Leu
145                 150                 155                 160

Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr
                165                 170                 175

Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser Gly Tyr Ser Tyr
            180                 185                 190

Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile
        195                 200                 205

Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala Arg Phe Ser Gly
    210                 215                 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His Pro Val Glu Glu
225                 230                 235                 240

Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ser Arg Glu Leu Pro Arg
                245                 250                 255

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Ala
            260                 265                 270

Arg Ala Pro Glu Met Pro Val Leu Glu Asn Arg Ala Ala Gln Gly Asp
        275                 280                 285

Ile Thr Ala Pro Gly Gly Ala Arg Arg Leu Thr Gly Asp Gln Thr Ala
    290                 295                 300

Ala Leu Arg Asp Ser Leu Ser Asp Lys Pro Ala Lys Asn Ile Ile Leu
305                 310                 315                 320

Leu Ile Gly Asp Gly Met Gly Asp Ser Glu Ile Thr Ala Ala Arg Asn
                325                 330                 335

Tyr Ala Glu Gly Ala Gly Gly Phe Phe Lys Gly Ile Asp Ala Leu Pro
            340                 345                 350

Leu Thr Gly Gln Tyr Thr His Tyr Ala Leu Asn Lys Lys Thr Gly Lys
        355                 360                 365

Pro Asp Tyr Val Thr Asp Ser Ala Ala Ser Ala Thr Ala Trp Ser Thr
    370                 375                 380

Gly Val Lys Thr Tyr Asn Gly Ala Leu Gly Val Asp Ile His Glu Lys
385                 390                 395                 400
```

-continued

```
Asp His Pro Thr Ile Leu Glu Met Ala Lys Ala Ala Gly Leu Ala Thr
                405                 410                 415

Gly Asn Val Ser Thr Ala Glu Leu Gln Asp Ala Thr Pro Ala Ala Leu
            420                 425                 430

Val Ala His Val Thr Ser Arg Lys Cys Tyr Gly Pro Ser Ala Thr Ser
        435                 440                 445

Glu Lys Cys Pro Gly Asn Ala Leu Glu Lys Gly Gly Lys Gly Ser Ile
    450                 455                 460

Thr Glu Gln Leu Leu Asn Ala Arg Ala Asp Val Thr Leu Gly Gly Gly
465                 470                 475                 480

Ala Lys Thr Phe Ala Glu Thr Ala Thr Ala Gly Glu Trp Gln Gly Lys
                485                 490                 495

Thr Leu Arg Glu Gln Ala Gln Ala Arg Gly Tyr Gln Leu Val Ser Asp
            500                 505                 510

Ala Ala Ser Leu Asn Ser Val Thr Glu Ala Asn Gln Gln Lys Pro Leu
        515                 520                 525

Leu Gly Leu Phe Ala Asp Gly Asn Met Pro Val Arg Trp Leu Gly Pro
    530                 535                 540

Lys Ala Thr Tyr His Gly Asn Ile Asp Lys Pro Ala Val Thr Cys Thr
545                 550                 555                 560

Pro Asn Pro Gln Arg Asn Asp Ser Val Pro Thr Leu Ala Gln Met Thr
                565                 570                 575

Asp Lys Ala Ile Glu Leu Leu Ser Lys Asn Glu Lys Gly Phe Phe Leu
            580                 585                 590

Gln Val Glu Gly Ala Ser Ile Asp Lys Gln Asp His Ala Ala Asn Pro
        595                 600                 605

Cys Gly Gln Ile Gly Glu Thr Val Asp Leu Asp Glu Ala Val Gln Arg
    610                 615                 620

Ala Leu Glu Phe Ala Lys Lys Glu Gly Asn Thr Leu Val Ile Val Thr
625                 630                 635                 640

Ala Asp His Ala His Ala Ser Gln Ile Val Ala Pro Asp Thr Lys Ala
                645                 650                 655

Pro Gly Leu Thr Gln Ala Leu Asn Thr Lys Asp Gly Ala Val Met Val
            660                 665                 670

Met Ser Tyr Gly Asn Ser Glu Glu Asp Ser Gln Glu His Thr Gly Ser
        675                 680                 685

Gln Leu Arg Ile Ala Ala Tyr Gly Pro His Ala Ala Asn Val Val Gly
    690                 695                 700

Leu Thr Asp Gln Thr Asp Leu Phe Tyr Thr Met Lys Ala Ala Leu Gly
705                 710                 715                 720

Asp Ile Ala His His His His His His
                725
```

<210> SEQ ID NO 95
<211> LENGTH: 969
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mAB #8860 scFv-leucine zipper fusion protein (miniantibody vector construct p8860-Zip#1.2)

<400> SEQUENCE: 95

```
atgaaatacc tattgcctac ggcagccgct ggattgttat tactcgcggc ccagccggcc     60

atggcggagg ttcagcttca gcagtctgga cctgagctgg tgaagcccgg ggcctcagtg    120
```

-continued

aagatttcct gcaaagcttc tggctacgca ttcagtagct cttggatgaa ctgggtgaag 180

cagaggcctg gacagggtct tgagtggatt ggacggattt atcctggaaa tggagatact 240

aactacaatg ggaagttcaa gggcaaggcc acactgactg cagacaaatc ctccagcaca 300

gcctacatgc agctcagcag cctgacctct gtggactctg cggtctattt ctgtgcagat 360

ggtaacgtat attactatgc tatggactac tggggtcaag gaacctcagt caccgtctcc 420

tcaggtggag gcggttcagg tgggcgcgcc tctggcggtg gcggatcgca aattgttctc 480

acccagtctc ctgcttcctt agctgtatct ctggggcaga gggccaccat ctcatgcagg 540

gccagcaaaa gtgtcagtac atctggctat agttatatgc actggtacca acagaaacca 600

ggacagccac ccaaactcct catctatctt gcatccaacc tagaatctgg ggtccctgcc 660

aggttcagtg gcagtgggtc tgggacagac ttcaccctca acatccatcc tgtggaggag 720

gaggatgctg caacctatta ctgtcagcac agtagggagc ttcctcggac gttcggtgga 780

ggcaccaagc tggaaatcaa acgggcggcc gcaccgaagc cttccactcc gcccgggtct 840

tcccgtatga aacagctgga agacaaagta gaggagctcc ttagcaagaa ctaccatcta 900

gaaaacgagg tagctcgtct gaaaaagctt gttggtgaac gtggtggtca ccatcaccat 960

caccattaa 969

<210> SEQ ID NO 96
<211> LENGTH: 322
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mAB #8860 scFv-leucine zipper fusion protein (miniantibody vector construct p8860-Zip#1.2)

<400> SEQUENCE: 96

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
1 5 10 15

Ala Gln Pro Ala Met Ala Glu Val Gln Leu Gln Gln Ser Gly Pro Glu
20 25 30

Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly
35 40 45

Tyr Ala Phe Ser Ser Ser Trp Met Asn Trp Val Lys Gln Arg Pro Gly
50 55 60

Gln Gly Leu Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asn Gly Asp Thr
65 70 75 80

Asn Tyr Asn Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys
85 90 95

Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Val Asp
100 105 110

Ser Ala Val Tyr Phe Cys Ala Asp Gly Asn Val Tyr Tyr Tyr Ala Met
115 120 125

Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly
130 135 140

Gly Ser Gly Gly Arg Ala Ser Gly Gly Gly Gly Ser Gln Ile Val Leu
145 150 155 160

Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr
165 170 175

Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser Gly Tyr Ser Tyr
180 185 190

Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile

-continued

```
        195                 200                 205

Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala Arg Phe Ser Gly
    210                 215                 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His Pro Val Glu Glu
225                 230                 235                 240

Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ser Arg Glu Leu Pro Arg
                245                 250                 255

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Pro
            260                 265                 270

Lys Pro Ser Thr Pro Pro Gly Ser Ser Arg Met Lys Gln Leu Glu Asp
        275                 280                 285

Lys Val Glu Glu Leu Leu Ser Lys Asn Tyr His Leu Glu Asn Glu Val
    290                 295                 300

Ala Arg Leu Lys Lys Leu Val Gly Glu Arg Gly Gly His His His His
305                 310                 315                 320

His His


<210> SEQ ID NO 97
<211> LENGTH: 270
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:part of
      plasmid pMycHis6 differing from vector pCOCK

<400> SEQUENCE: 97

caggaaacag ctatgaccat gattacgcca agcttccatg aaaattctat ttcaaggaga     60

cagtcataat gaaataccta ttgcctacgg cagccgctgg attgttatta ctcgcggccc    120

agccggccat ggcccaggtg cagctgcagg cgcgcctgca ggtcgacctc gagatcaaac    180

gggcggccgc agaacaaaaa ctcatctcag aagaggatct gaatggggcg gcacatcacc    240

atcaccatca ctaataagaa ttcactggcc                                     270


<210> SEQ ID NO 98
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:part of
      plasmid pMycHis6 differing from vector pCOCK

<400> SEQUENCE: 98

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
  1               5                  10                  15

Ala Gln Pro Ala Met Ala Gln Val Gln Leu Gln Ala Arg Leu Gln Val
            20                  25                  30

Asp Leu Glu Ile Lys Arg Ala Ala Ala Glu Gln Lys Leu Ile Ser Glu
        35                  40                  45

Glu Asp Leu Asn Gly Ala Ala His His His His His His
    50                  55                  60


<210> SEQ ID NO 99
<211> LENGTH: 888
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:198AB2 scFv
      linked to c-myc-tag and His6 tag (ORF of expression vector
      pMycHis6-198AB2#102)
<220> FEATURE:
```

-continued

<221> NAME/KEY: modified_base
<222> LOCATION: (228)
<223> OTHER INFORMATION: n = g, a, c or t

<400> SEQUENCE: 99

atgaaatacc tattgcctac ggcagccgct ggattgttat tactcgcggc ccagccggcc 60

atggccgagg tgaagctggt ggagtctggg ggaggcttag tgaagcctgg agggtccctg 120

aaactctcct gtgcagcctc tggattcact ttcagtagct ataccatgtc ttgggttcgc 180

cagactccgg agaagaggct ggagtgggtc gcaaccatta gtagtggngg tagttccacc 240

tactatccag acagtgtgaa gggccgattc accatctcca gagacaatgc caagaacacc 300

ctgtacctgc aaatgagcag tctgaggtct gaggacacag ccatgtatta ctgtacaaga 360

gaggggggtg gtttcaccgt caactggtac ttcgatgtct ggggcgcagg aacctcagtc 420

accgtctcct caggtggagg cggttcaggt gggcgcgcct ctggcggtgg cggatcggac 480

attgtgctga cacagtctcc agcttctttg gctgtgtctc tagggcagag ggccaccata 540

tcctgcagag ccagtgaaag tgttgatagt tatggctata attttatgca ctggtatcag 600

cagataccag gacagccacc caaactcctc atctatcgtg catccaacct agagtctggg 660

atccctgcca ggttcagtgg cagtgggtct aggacagact tcaccctcac cattaatcct 720

gtggaggctg atgatgttgc aacctattac tgtcagcaaa gtaatgagga tccgctcacg 780

ttcggtactg ggaccagact ggaaataaaa cgggcggccg cagaacaaaa actcatctca 840

gaagaggatc tgaatggggc ggcacatcac catcaccatc actaataa 888

<210> SEQ ID NO 100
<211> LENGTH: 294
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:198AB2 scFv linked to c-myc-tag and His6 tag (ORF of expression vector pMycHis6-198AB2#102)

<400> SEQUENCE: 100

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
1 5 10 15

Ala Gln Pro Ala Met Ala Glu Val Lys Leu Val Glu Ser Gly Gly Gly
20 25 30

Leu Val Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly
35 40 45

Phe Thr Phe Ser Ser Tyr Thr Met Ser Trp Val Arg Gln Thr Pro Glu
50 55 60

Lys Arg Leu Glu Trp Val Ala Thr Ile Ser Ser Gly Gly Ser Ser Thr
65 70 75 80

Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
85 90 95

Ala Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Arg Ser Glu Asp
100 105 110

Thr Ala Met Tyr Tyr Cys Thr Arg Glu Gly Gly Gly Phe Thr Val Asn
115 120 125

Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr Ser Val Thr Val Ser Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Arg Ala Ser Gly Gly Gly Gly Ser Asp
145 150 155 160

Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln

-continued

```
                165                 170                 175

Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Ser Tyr Gly
            180                 185                 190

Tyr Asn Phe Met His Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro Lys
        195                 200                 205

Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Ile Pro Ala Arg
    210                 215                 220

Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Asn Pro
225                 230                 235                 240

Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Glu
                245                 250                 255

Asp Pro Leu Thr Phe Gly Thr Gly Thr Arg Leu Glu Ile Lys Arg Ala
            260                 265                 270

Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Gly Ala Ala
        275                 280                 285

His His His His His His
    290
```

<210> SEQ ID NO 101
<211> LENGTH: 876
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mAB #8860 scFv linked to c-myc-tag and His6-tag designated 8860-M/H#4c (plasmid vector p8860-M/H#4c)

<400> SEQUENCE: 101

```
atgaaatacc tattgcctac ggcagccgct ggattgttat tactcgcggc ccagccggcc     60

atggccgagg ttcagcttca gcagtctgga cctgagctgg tgaagcccgg ggcctcagtg    120

aagatttcct gcaaagcttc tggctacgca ttcagtagct cttggatgaa ctgggtgaag    180

cagaggcctg gacagggtct tgagtggatt ggacggattt atcctggaaa tggagatact    240

aactacaatg ggaagttcaa gggcaaggcc acactgactg cagacaaatc ctccagcaca    300

gcctacatgc agctcagcag cctgacctct gtggactctg cggtctattt ctgtgcagat    360

ggtaacgtat attactatgc tatggactac tggggtcaag gaacctcagt caccgtctcc    420

tcaggtggag gcggttcagg tgggcgcgcc tctggcggtg gcggatcgca aattgttctc    480

acccagtctc ctgcttcctt agctgtatct ctggggcaga gggccaccat ctcatgcagg    540

gccagcaaaa gtgtcagtac atctggctat agttatatgc actggtacca acagaaacca    600

ggacagccac ccaaactcct catctatctt gcatccaacc tagaatctgg ggtccctgcc    660

aggttcagtg gcagtgggtc tgggacagac ttcaccctca acatccatcc tgtggaggag    720

gaggatgctg caacctatta ctgtcagcac agtagggagc ttcctcggac gttcggtgga    780

ggcaccaagc tggaaatcaa acgggcggcc gcagaacaaa aactcatctc agaagaggat    840

ctgaatgggg cggcacatca ccatcaccat cactaa                              876
```

<210> SEQ ID NO 102
<211> LENGTH: 291
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:mAB #8860 scFv linked to c-myc-tag and His6-tag designated 8860-M/H#4c (plasmid vector p8860-M/H#4c)

<400> SEQUENCE: 102

-continued

```
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
  1               5                  10                  15

Ala Gln Pro Ala Met Ala Glu Val Gln Leu Gln Gln Ser Gly Pro Glu
             20                  25                  30

Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly
         35                  40                  45

Tyr Ala Phe Ser Ser Ser Trp Met Asn Trp Val Lys Gln Arg Pro Gly
     50                  55                  60

Gln Gly Leu Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asn Gly Asp Thr
 65                  70                  75                  80

Asn Tyr Asn Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys
                 85                  90                  95

Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Val Asp
            100                 105                 110

Ser Ala Val Tyr Phe Cys Ala Asp Gly Asn Val Tyr Tyr Tyr Ala Met
        115                 120                 125

Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly
    130                 135                 140

Gly Ser Gly Gly Arg Ala Ser Gly Gly Gly Gly Ser Gln Ile Val Leu
145                 150                 155                 160

Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr
                165                 170                 175

Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Ser Gly Tyr Ser Tyr
            180                 185                 190

Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile
        195                 200                 205

Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala Arg Phe Ser Gly
    210                 215                 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His Pro Val Glu Glu
225                 230                 235                 240

Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ser Arg Glu Leu Pro Arg
                245                 250                 255

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Glu
            260                 265                 270

Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Gly Ala Ala His His His
        275                 280                 285

His His His
    290
```

<210> SEQ ID NO 103
<211> LENGTH: 74
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:annealed oligonucleotide

<400> SEQUENCE: 103

```
ggccgcagaa caaaaactca tctcagaaga ggatctgaat ggggcggcac atcaccatca     60

ccatcactaa taag                                                       74
```

<210> SEQ ID NO 104
<211> LENGTH: 69
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Description of Artificial Sequence:annealed oligonucleotide

<400> SEQUENCE: 104

ttattagtga tggtgatggt gatgtgccgc cccattcaga tcctcttctg agatgagttt 60

ttgttctgc 69

<210> SEQ ID NO 105
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:CDR3 peptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(16)
<223> OTHER INFORMATION: Xaa = any amino acid

<400> SEQUENCE: 105

Cys Xaa Xaa Tyr Gly Asn Ser Pro Lys Gly Phe Ala Tyr Xaa Xaa Cys
1 5 10 15

<210> SEQ ID NO 106
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:CDR3 peptide

<400> SEQUENCE: 106

Phe Arg Asn Arg Gly Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp
1 5 10 15

<210> SEQ ID NO 107
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:portion of plasmid pMycHis6 with pelB-leader, polylinker and c-myc tag

<400> SEQUENCE: 107

Leu Ala Ala Gln Pro Ala Met Ala Gln Val Gln Leu Gln Ala Arg Leu
1 5 10 15

Gln Val Asp Leu Glu Ile Lys Arg Ala Ala Ala Glu Gln Lys
20 25 30

<210> SEQ ID NO 108
<211> LENGTH: 90
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:portion of plasmid pMycHis6 with pelB-leader, polylinker and c-myc tag

<400> SEQUENCE: 108

ctcgcggccc agccggccat ggcccaggtg cagctgcagg cgcgcctgca ggtcgacctc 60

gagatcaaac gggcggccgc agaacaaaaa 90

<210> SEQ ID NO 109
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:c-myc-tag

-continued

<400> SEQUENCE: 109

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Gly
1 5 10

<210> SEQ ID NO 110
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:His6-tag

<400> SEQUENCE: 110

His His His His His His
1 5

<210> SEQ ID NO 111
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:flexible linker

<400> SEQUENCE: 111

Gly Gly Gly Gly Ser Gly Gly Arg Ala Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> SEQ ID NO 112
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:A1 peptide core sequence

<400> SEQUENCE: 112

Glu Gly Gly Gly Tyr Tyr Val Asn Trp Tyr Phe Asp
1 5 10

What is claimed is:

1. An isolated antibody or antibody fragment thereof that binds Factor IX or Factor IXa and increases the procoagulant activity of Factor IXa.

2. The antibody or antibody fragment according to claim **1** that increases the procoagulant activity of Factor IXa in the presence of Factor VIII inhibitors.

3. The antibody or antibody fragment according to claim **1** wherein the antibody is an IgG, IgM, IgA or IgE antibody.

4. The antibody or antibody fragment according to claim **1**, wherein said antibody or antibody fragment is selected from the group consisting of a monoclonal antibody, a chimeric antibody, a humanized antibody, a single chain antibody, a bispecific antibody, a diabody, and di-, oligo- or multimers thereof.

5. A CDR3 peptide of the antibody or antibody fragment according to claim **1** consisting of an amino acid sequence selected from the group consisting of:

Tyr-Gly-Asn-Ser-Pro-Lys-Gly-Phe-Ala-Tyr (SEQ ID NO:5); and

Asp-Gly-Gly-His-Gly-Tyr-Gly-Ser-Ser-Phe-Asp-Tyr (SEQ ID NO:6).

6. The antibody or antibody fragment according to claim **1**, wherein the variable region of said antibody or antibody fragment comprises amino acids 1–119 and amino acids 135–242 as listed in SEQ ID NO:82.

7. The antibody or antibody fragment according to claim **6** that additionally comprises an artificial linker sequence.

8. The antibody or antibody fragment according to claim **1**, wherein the variable region of said antibody or antibody fragment comprises amino acids 1–121 and amino acids 137–249 as listed in SEQ ID NO:84.

9. The antibody or antibody fragment according to claim **8** that additionally comprises an artificial linker sequence.

10. The antibody or antibody fragment according to claim **1**, wherein the variable region of said antibody or antibody fragment comprises amino acids 1–122 and amino acids 138–249 as listed in SEQ ID NO:86.

11. The antibody or antibody fragment according to claim **10** that additionally comprises an artificial linker sequence.

12. A hybridoma cell line secreting an antibody that binds Factor LX or Factor IXa and increases the procoagulant activity of Factor IXa.

13. The hybridoma cell line according to claim **12** that is selected from the group consisting of cell lines having ECACC deposit numbers 99090924, 99090925, 99090926, 99121614, 99121615, 99121616, 99121617, 99121618, 99121619 and 99121620.

14. An antibody that is secreted by a hybridoma cell line according to claim **12**.

15. A preparation comprising an antibody or antibody fragment according to claim **1** and a pharmaceutically acceptable carrier.

16. The preparation according to claim 15, additionally comprising Factor IXaα and/or Factor IXaβ.

17. A method of obtaining an antibody that interacts with Factor IX or Factor IXa and increases the procoagulant activity of Factor IXa, comprising the steps of:

immunizing an immunocompetent mouse with an antigen selected from the group consisting of FIX, FIXaα, FIXaβ or fragments thereof,

isolating spleen cells of the immunized mouse,

producing hybridoma cells,

screening the hybridoma cell supernatants for an increase in the procoagulant activity of Factor IXa, isolating and purifying the antibody from a supernatant from the hybridoma cells which exhibit an increase in the procoagulant activity of Factor IXa.

18. The antibody or antibody fragment according to claim 4, wherein the antibody fragment is a single chain antibody.

19. The antibody or antibody fragment according to claim 4, wherein the antibody is a humanized antibody.

20. The antibody or antibody fragment according to claim 2 wherein the antibody is selected from the group consisting of an IgG, IgM, IgA or IgE antibody.

21. The antibody or antibody fragment of claim 1, wherein the antibody fragment comprises a CDR3 peptide.

22. The antibody or antibody fragment of claim 1, wherein the antibody fragment is a CDR3 peptide.

* * * * *

CERTIFICATE OF COMPLIANCE WITH RULE 32(A)

1. This brief complies with the type-volume limitations of Federal Circuit Rule 32(b)(1) because this brief contains 12,922 words, including the words in images and excluding the parts of the brief exempted by Federal Rule of Appellate Procedure 32(f), Federal Circuit Rule 32(b)(2), and Federal Circuit Rule 28(a)(12).

2. This brief complies with the typeface requirements of Federal Rule of Appellate Procedure 32(a)(5) and the type-style requirements of Federal Rule of Appellate Procedure 32(a)(6) because this brief has been prepared in a proportionally spaced typeface using Microsoft Word 360 in Times New Roman 14 point font.

/s/ William R. Peterson
William R. Peterson
Counsel for Appellants,
Baxalta Inc. and Baxalta GmbH

Dated: June 10, 2022